PREPARATION OF NOVEL HETEROCYCLIC-RING ANALOGUFS OF BIOLF-62; APPLICATION OF ^{29}SI NMR TO NUCLEOSIDES AND THE INVESTIGATION OF 2,4-DINITROBENZENESULFENYL AS A PROTECTING GROUP FOR RIBONUCLEOTIDE SYNTHESIS

A Thesis

by

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To my Mother,

Emily MacAskill

Chemistry Ph.D.

PREPARATION OF NOVEL HETEROCYCLIC-RING ANALOGUES OF BIOLF-62; APPLICATIONS OF ^{29}SI NMR TO NUCLEOSIDES AND THE INVESTIGATION OF 2,4-DINITROBENZENESULFENYL AS A PROTECTING GROUP FOR RIBONUCLEOTIDE SYNTHESIS.

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Abstract

In light of the remarkable antiviral activity of acyclic nucleoside analogues such as that of BIOLF-62 against the herpes viruses, a number of products in which heterocyclic bases were coupled to the active acyclic sugar moiety were prepared and submitted for biological testing.

Various dimethoxytritylated and t-butyldimethylsilylated derivatives of arabinoadenosine were prepared and fully characterised by ^{1}H and ^{13}C NMR spectroscopy. ^{29}Si INEPT as well as ^{29}Si-^{1}H correlated NMR were used to study various t-butyldimethylsilyl and triisopropylsilyl substituted ribonucleosides.

In an effort aimed at the development of new and better nucleoside protecting functions, the 2,4-dinitrobenzenesulfenyl group which is stable to both acidic and basic conditions, was used for 5'-hydroxyl protection of ribonucleosides and its compatibility with the phosphodichoridite nucleoside coupling procedure was investigated. The nitrobenzenesulfenyl group was used in conjunction with the dimethoxytrityl group for 2'-hydroxyl protection in the synthesis of a UpU dimer. The latter was fully characterised by enzymatic degradation and HPLC analysis of the products.

Chimie Ph.D.

PREPARATION DE NOUVEAUX ANALOGUES ACYCLIQUES DU BIOLF-62; APPLICATIONS DE LA RMN DU ^{29}SI AUX NUCLEOSIDES ET L'INVESTIGATION DU DINITRO-2,4 BENZENESULFENYLE COMME GROUPEMENT PROTECTEUR DANS LA SYNTHESE D'OLIGORIBONUCLEOTIDES

par

Suzanne Boisvert

Résumé

Suite à l'activité antivirale remarquable de certains analogues de nucléosides telle celle du BIOLF-62 contre les virus de l'hèrpès, quelques produits résultant du couplage de bases hétérocycliques à la portion acyclique active furent préparés pour soumission à des essais biologiques.

L'intérêt biologique des arabinonucléosides a inspiré la préparation de dérivés diméthoxytritylés et t-butyldiméthylsilylés de l'arabinoadénosine. Ces produits furent complètement caractérisés par spectroscopie RMN du ^{1}H et ^{13}C. La RMN INEPT du ^{29}Si et hétéro-corrélée ^{29}Si-^{1}H furent employées dans l'étude d'une série de ribonucléosides t-butyldiméthylsilylés et triisopropylsilylés.

Dans un effort visant au développement de nouveaux groupements protecteurs, le dinitro-2,4 benzènesulfényle qui est à la fois stable en milieu basique et acide, fut employé pour fonctionaliser l'hydroxyle en position 5' du ribonucléoside et sa compatibilité avec la méthode du phosphodichloridure pour la synthèse d'acides nucléiques fut vérifiée. Ce groupement a été employé en conjonction avec le diméthoxytrityle en position 2' lors de la synthèse du dinucléotide UpU. Ce produit fut caractérisé par dégradation enzymatique et analyse des résidus par CLHP.

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List of Abbreviations

A	adenosine
Ac	acetyl
Ara	arabino
Bn	benzyl
b.p.	boiling point
bs	broad singlet
BSA	benzenesulfonic acid
Bz	benzoyl
C	cytidine
CE	cyanoethyl
d	doublet
dd	doublet of doublets
dt	doublet of triplets
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DEPT	distorsionless enhancement by polarisation transfer
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMT	dimethoxytrityl
DNA	deoxyribonucleic acid
DNBS	2,4-dinitrobenzenesulfenyl
Et	ethyl
EtOAc	ethyl acetate
eq.	equivalent
FT	Fourier transform

G	guanosine
H	hypoxanthine
HETCOR	hetero-correlated
HSV	Herpes Simplex Virus
Hz	Hertz
INEPT	insensitive nuclei enhancement by polarisation transfer
J	coupling constant
Lit.	literature
Lv	levulinyl
M	molar
m	multiplet
MeOH	methanol
mmol.	millimole
MMT	monomethoxytrityl
m.p.	melting point
MS	mass spectrum
NBS	2-nitrobenzenesulfenyl
NMR	nuclear magnetic resonance
O.D.	optical density. One O.D. unit is the amount of material which produces an absorbance of 1.0 at 260 nm when dissolved in 1 mL in a 1 cm cell.
PE	petroleum ether
Ph	phenyl
Pyr.	pyridine
q	quartet
qi	quintet
R_f	relative mobility
RNA	ribonucleic acid

R.T.	room temperature
s	singlet
T	thymidine
t	triplet
TBAF	tetra-n-butylammonium fluoride
TBDMS	t-butyldimethylsilyl
TCE	trichloroethyl
THF	tetrahydrofuran
THP	tetrahydropyranyl
TiPDS	tetraisopropyldisiloxyl
TiPS	triisopropylsilyl
TK	thymidine kinase
TLC	thin layer chromatography
TMS	trimethylsilyl
TPS	2,4,6-triisopropylbenzenesulfonyl
Trac	triphenylmethoxyacetyl
U	uridine
UV	ultraviolet

INTRODUCTION

The history of nucleic acids, recent focus of much interest, began in the mid nineteenth century with a discovery by Miescher [1, 2]. While attempting to isolate the nuclei from the white blood cells of human pus, he extracted an unknown nitrogen and phosphorous containing material which he named nuclein. He later identified this material as a "polybasic" acid, the properties of which he attributed to the presence of a phosphoric acid component. The isolated substance was in fact a depolymerised form of deoxyribonucleic acid (DNA).

A link between nuclein and heredity was unsuspected when in 1865, Mendel began experiments which established the basic principles of classical genetics i.e. the transmission from one generation to the next of genetic elements and the expression of phenotypes [2]. Over the next decade, the mechanisms of fertilisation and cell division were the focus of much research [3]. As a result of this, in 1883, Van Beneden realised that continuity in both these processes depended on chromosomes, threadlike particles visible in the nucleus during cell division [3]. It became apparent that an understanding of fertilisation was at the same time an understanding of heredity. Although Zacharias had already shown that the material of chromosomes was either nuclein or intimately associated with it, Von Sachs (1882) and Hertwig (1884) were the first to suggest and recognise nuclein as the genetic material [3]. The rediscovery of Mendel's "units" of heredity and their linking with genes, by Morgan in the early twentieth century,

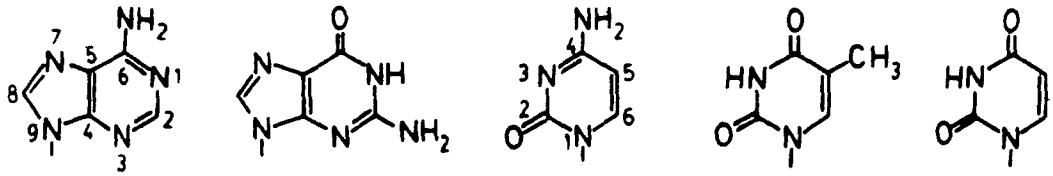
confirmed the idea, originally expressed in 1884 by Weismann, that chromosomes transmit heredity [3]. However, it was not until the late 1940's following independant work by groups in France and the United States [4] that DNA was recognised as the molecular basis for heredity [5].

Structure elucidation by partial hydrolysis of nucleic acid had already established the presence of phosphoric acid as well as sugar and nitrogenous base components when, circa 1908, two distinct nucleic acids (one present within the nucleus and the other, mostly in the cytoplasm) were identified. In 1918, researchers at the Rockefeller Institute isolated the five carbon β-D-sugar of the cytoplasmic nucleic acid and named it ribose. The 2'-deoxyribose present in the nucleus was later isolated by the same group [6]. The five derivatives of purine and pyrimidine found to be the major nitrogen constituents of deoxyribonucleic (DNA) and ribonucleic acids (RNA) are shown in Figure 1. These are adenine (1), guanine (2), cytosine (3), thymine (4) and uracil (5).

Figure 1

The Common Heterocyclic Bases of Nucleic Acids

Purines Pyrimidines



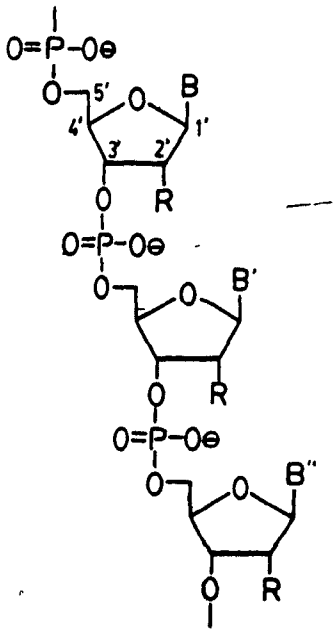
1 2 3 4 5

The primary structure of nucleic acids was determined in the 1950's to be a high polymer of nucleoside units joined from the 3' to the 5' positions by phosphate diester linkages (Figure 2). Nucleosides consist of a

pyrimidine or purine base bound to the C-1' position of the sugar ring by a β-N-glycosidic linkage. The purines are linked by the N-9 position to form adenosine (A) and guanosine (G) while the pyrimidines are linked by the N-1 position to form cytidine (C), thymidine (T) and uridine (U).

Figure 2

The Primary Structures of DNA and RNA



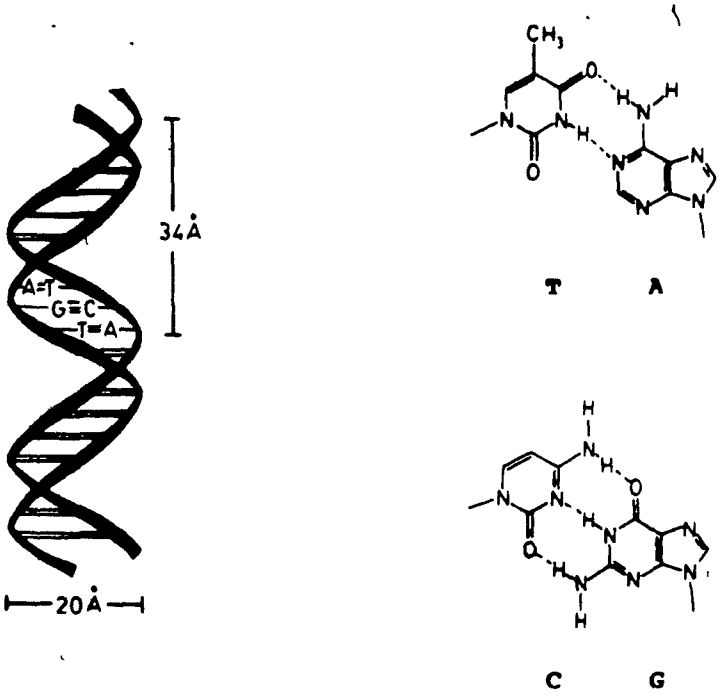
	R	B, B', B"
DNA	H	1, 2, 3, 4
RNA	OH	1, 2, 3, 5

In 1950, Wilkins and Franklin described two forms of DNA, A and B, which differ in their degree of hydration but it was not until 1953 that Watson and Crick proposed a model for the secondary structure of B-DNA [7]. This was based on the X-ray diffraction data of Wilkins and Franklin as well as the base equivalence studies of Chargaff. The structure, reproduced in Figure 3, consists of two antiparallel polynucleotide chains interwound in the form of a right handed double helix. The bases of each strand are projected toward the center and form parallel complementary base pairs stabilised by two (A-T) or three (G-C) hydrogen bonds [8].

The helix is of constant diameter as the base pairs each consist of a large purine and small pyrimidine residue.

Figure 3

Base Pairing and the Secondary Structure of B-DNA



In the late 1970's, a left handed helix for which there is, as yet, no direct evidence for existence within cells, was obtained in crystalline state [9]. This less stable Z-DNA takes the form of a narrower helix than the Watson and Crick B-DNA and is traced by a single groove [10, 11] whereas B-DNA is furrowed by both a minor and a major groove. The bases are reoriented ("flipped") in this structure and number twelve per turn versus the ten base pairs in B-DNA. The biological significance of Z-DNA is still elusive though it has been linked with the control regions of viral DNA.

RNA generally exists as a single polynucleotide chain rather than a double helix of antiparallel strands. However, base pairing can take place

within and between RNA molecules. Certain forms of double stranded replicating viral RNA possess a similar degree of regularity as B-DNA [12], but base pair hydrogen bonding usually occurs within the same strand causing certain regions to adopt a helix or pseudo-helix conformation thus forming hairpin loops. This structure differs from the DNA helix due to the presence of a 2'-hydroxyl group in the ribose phosphate backbone [13]. It counts approximately eleven base pairs per turn and posseses both a minor and a major groove.

Nucleic acid macromolecules are present in all living cells, existing either in a free form or associated with proteins. Even the most simple viruses are composed of a nucleic acid molecule surrounded by a protein shell and viroids consist of an RNA molecule only. Although RNA stores genetic information in most plant viruses and some animal viruses, this function is assumed by DNA in eucaryotic cells (cells which possess a nucleus). The biological structures (RNA molecules contain a greater number of modified nitrogenous bases) and roles of RNA in the expression of genetic information are more diverse [14]. Transfer RNA (tRNA) transports a specific amino acid during protein synthesis. Messenger RNA (mRNA), a short lived species, translates the information for protein synthesis. Heterogeneous nuclear RNA (hnRNA), a precursor to mRNA, exists only in the nucleus where 90% is degraded. Ribosomal RNA (rRNA), which accounts for 80% of cellular RNA, combines with protein to form ribosomes, the sites of protein synthesis in the cell.

The close linking of nucleic acids with cellular processes is the key to their usefulness in the understanding of structure to function relationships and in the enhancement of activity in deficient biological systems. It is also to this narrow link that the constituent units of

nucleic acids, the nucleosides, and their derivatives, owe their remarkable therapeutic and diagnostic properties [15].

Nucleosides and their Analogues as Antiviral Agents

The search for antiviral compounds has been an extremely difficult one because viruses are physiologically inert outside their host cell where they are incapable of growth, reproduction, response to external stimuli, independant metabolism or mobility [16]. Once invasion of the cell has occurred, the viral nucleic acid and protein are so intimately integrated to the host cell metabolism that any procedure which interferes with viral activity inevitably interferes with the host's as well.

Viruses have alternately been described as exceptionally complex molecules or exceptionally simple microbes. They are pathogens of plants, bacteria, insects and animals, ranging in size from 10 to 250 nm and are responsible for 60% of the diseases occurring in developed countries [17]. Viruses are nucleoproteins consisting of nucleic acid, usually one molecule of DNA or RNA which confers infective capability, and a protein coat or capsid. This protective shield is composed of protein subunits (capsomeres) in an orderly arrangement which are capable of reaggregation. Enveloped viruses also possess an external lipid or polysaccharide layer usually derived from the host cell membrane.

Viroids, discovered in the early 1970's, are the simplest known agents of infectious disease [18-20]. Either forerunners or degenerate versions of viruses, these agents cause several diseases of higher plants. Viroids consist of a small single stranded circular RNA molecule (ca 360

nucleotides) which adopts a rodlike secondary stucture. They differ from viruses by the absence of a protein coat.

Among DNA viruses, the herpes group is of particular interest as they are the cause of the most common viral diseases in man [21]. Infections due to herpes viruses have been known for over 25 centuries. The herpes simplex viruses of type I and II (HSV-I, HSV-II) are responsible for cold sores, encephalitis as well as eye and genital infections. Other viruses of this group cause afflictions ranging from chicken pox to mononucleosis (Table I). The herpes viruses are interesting in that they undergo a period of dormancy in ganglionic sites or lymphocytes [21, 22]. For reasons unknown, usually related to stress factors, the latent virus can be reactivated to initiate a recurrent or a new form of illness.

TABLE I

Herpes Infections in Humans

HSV-I, HSV-II	cold sores encephalitis eye infections
Varicella Zoster	chicken pox shingles
Cytomegalovirus	pneumonia CNS diseases*
Epstein Barr Virus	mononucleosis

*CNS: Central Nervous System

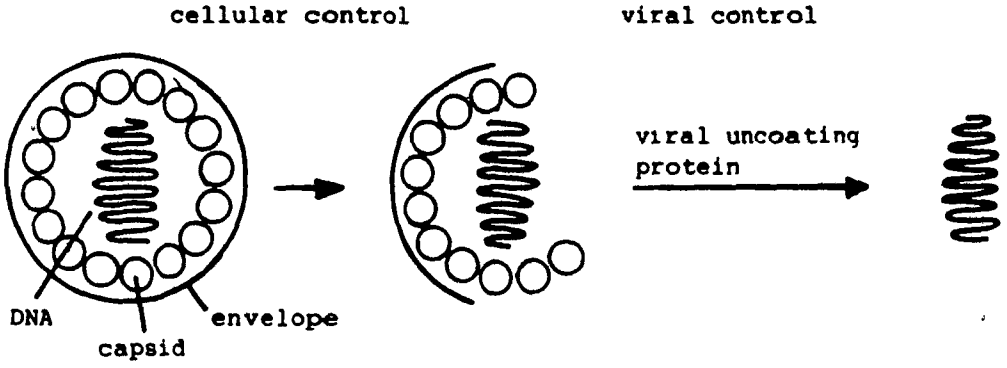
The herpes simplex virus is an eicosahedron (twenty sided structure) ranging in diameter from 150 to 250 nm [23]. An idea of the virus' relative dimension can be obtained by comparison with a bacterium such as the typhoid bacillus which has a diameter of 1000 nm. The herpes simplex virus is a non covalently associated supramolecular system consisting of a

complementary, double stranded DNA core surrounded by 162 capsomeres. The capsid is surrounded by a three layered envelope bearing spikes ranging in length between 8 to 10 nm and thought to be virus-specific glycoproteins.

In the first stage of herpes infection [24], the cell surface interacts with that of the virus resulting in the latter's fusion with the host's plasma membrane. Then follows a period of eclipse (second stage), determined by the rate of viral uncoating (Figure 4). Cellular enzymes first begin the degradation of the viral capsid which is later continued by an uncoating protein produced by the viral DNA (vDNA). The naked vDNA is thought to combine to cellular protein to evade the action of hydrolases. In the case of herpes infections, the virus is transported into the cell nucleus before completion of the uncoating.

Figure 4

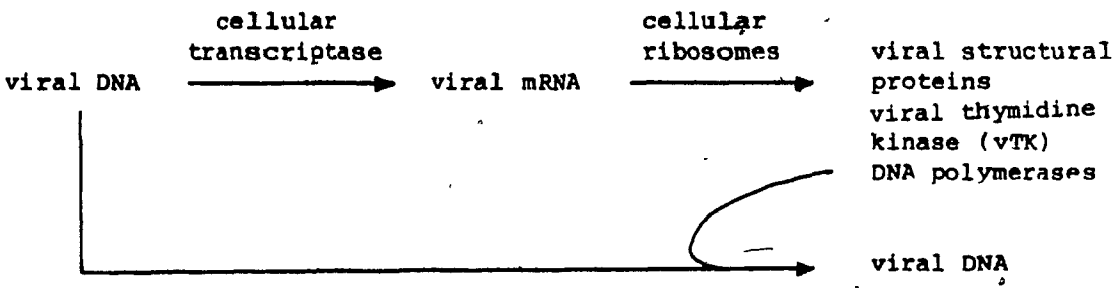
The Viral Uncoating Process



In the third stage of infection, the virus monopolises the biosynthetic machinery of the cell, reprogramming the host cell ribosomes to redirect protein synthesis. Moreover, the synthesis of the host DNA is inhibited by action of a deoxyphosphatase enzyme, initiated either by the viral genome (ensemble of genes) or by activation of a latent host cell control mechanism. The expression of viral genes occurs during the fourth

stage of infection. Viral DNA serves as template for the transcription of viral mRNA and production of viral proteins. Both processes are dependant upon the host cell for the supply of low molecular weight precursors (nucleosides and amino acids). Viruses usually possess a single genome coding for a single mRNA molecule. The viral mRNA must be segmented by proteolytic enzymes to accomodate the protein synthesis mechanism of eucaryotic cells, which require monocistronic mRNA (mRNA which codes for one protein). At the fifth stage, viral DNA is replicated by the viral polymerase produced. These steps are represented in a simplified form in Scheme I.

Scheme I



The penultimate stage consists in the maturation of the viruses. This is a process of self assembly during which nucleic acid inclusion occurs following aggregation of the capsomeres. The final stage is usually cell lysis and release of the viruses, however, in the case of herpes viruses this pathway is aborted to cause a persistent infection. Partially formed nucleoproteins accumulate in the cell. Some viruses such as the oncogenic pathogens, cause a third form of infection, cell transformation. The three are mutually exclusive.

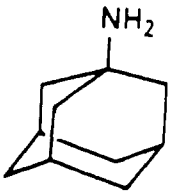
There are a number of stages at which chemotherapeutic or chemoprophylactic agents may exert their action. Antiviral compounds may block or

kill the virus before it enters the cell and causes infection. Alternatively, they may prevent the attachment to, or the penetration of the cell. However, the most effective agents enter the cell and prevent viral multiplication either by interrupting the synthesis of viral protein or nucleic acid or, by causing a mutation that results in the production of non infectious or immature viral particles. Because the virally induced or encoded enzymes necessary for multiplication usually act more rapidly than the equivalent cellular catalysts, the most successful antiviral agents exhibit a specificity for these enzymes with minimal disruption of the host's biochemical processes. In order to be effective, these agents must also be readily soluble in aqueous media, able to cross cellular membranes and cause minimal immunosuppressive, teratogenic, mutagenic and carcinogenic effects.

The development of effective antiviral compounds has been less successful than that of antibacterial agents due to the limited number of target virus specific enzymes. Nevertheless several agents, mostly nucleoside analogues, possess significant activity against viruses though few have been approved for the treatment of human infections [17, 21, 22, 25-28].

Among the non nucleoside agents, the symmetrical amine adamantamine or amantadine (6) is noteworthy. This agent is used against certain strains of influenza A, an RNA virus [29]. Adamantamine, first approved for systemic use in the United States in 1966, was originally thought to be a prophylactic agent which blocked viral penetration of the host cell [16]. More recently, its curative action was established [30, 31]. After the virus has entered the cell, amantadine appears to inhibit viral transcription at an early stage, between the uncoating process and viral RNA synthesis.

Arildone (7) and a water soluble pyrazole derivative (8) selectively inhibit the replication of some RNA and DNA viruses by blocking viral uncoating thus preventing takeover of protein synthesis [32]. Arildone is active against certain strains of polio virus and against the HSV family. At the concentrations needed for activity, this agent does not adversely affect healthy cells [33]. Several sugar derivatives and modified polysaccharides possess significant activity against both DNA and RNA viruses. These antiviral agents interfere with the replication of enveloped viruses by incorporation into their glycoproteins and glycolipids thus disrupting the viral envelope. Accompanying cytotoxic effects are minimal. 2-Deoxy-D-glucose (9), used topically in the treatment of HSV infections [34], and D-glucosamine (10) are among the more effective agents from this group [25, 26].



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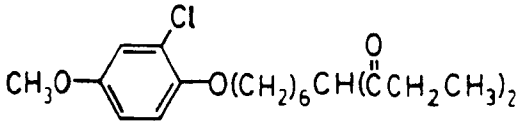

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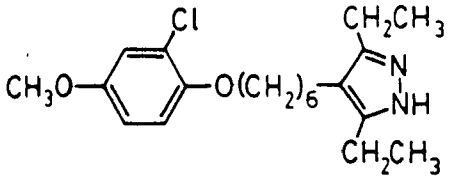

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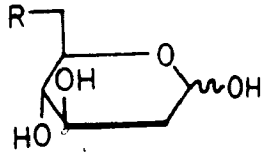
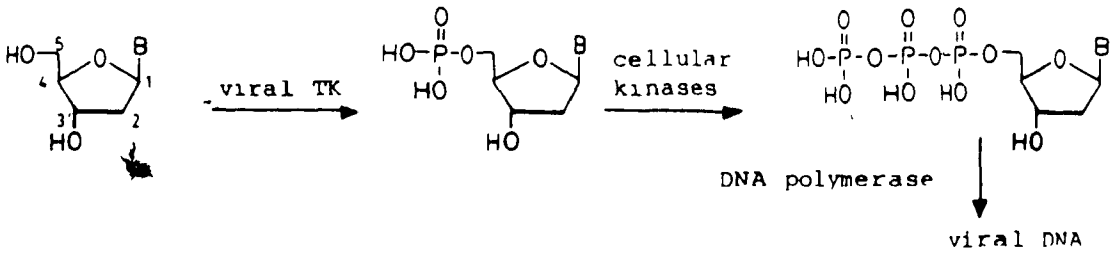


	R
9	OH
10	NH_2

More recently, several pyrimidine nucleoside derivatives have been useful in antiviral chemotherapy particularly against the herpes viruses [35]. The general mechanism for the action of these analoques against DNA pathogens is now understood to be the following (Scheme II). The agent is first phosphorylated by the virally induced, poorly selective thymidine kinase (TK). Cellular kinases subsequently convert the monophosphorylated agent to the triphosphate form which then acts as a substrate or competitive inhibitor of viral DNA polymerase. The incorporation of fraudulent nucleosides into viral DNA molecules may terminate or delay chain elongation or may cause distortions of the secondary and tertiary structures of the DNA.

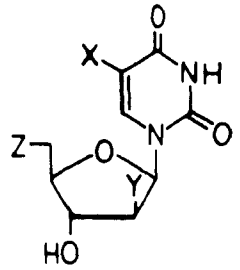
Scheme II



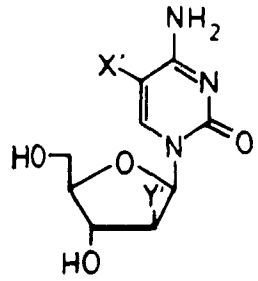
5-iodo-2'-deoxyuridine (IDU) (11) and other halogenated pyrimidine nucleosides such as 12, cause the production of faulty viral DNA as does the trifluorothymidine derivative (TFT) (13) [36, 37]. Their use is limited by their systemic toxicity and low antiviral specificity as they are incorporated into DNA (in the place of thymidine) in both virus infected and non-infected cells [26]. 5'-Amino-5-iodo-2',5'-dideoxyuridine (14) is active against both HSV strains [38-40]. This agent is preferentially phosphorylated by viral TK and the phosphoramidate formed is incorporated only into viral DNA thus exhibiting lower host cell toxicity. 5-Ethyl-2'-

deoxyuridine (EDU) (15) and 5-propyl-2'-deoxyuridine (PDU) (16) are specifically phosphorylated by HSV TK [26, 41]. Although both may be incorporated into host and viral DNA, PDU inhibits the induction of viral DNase and DNA polymerase. E-5-Bromovinyl-2'-deoxyuridine (BVDU), 17, has proved considerably more effective than the aforementioned agents and exhibits lower cell toxicity [42]. It is specifically phosphorylated in the 5' position by HSV-I and HSV-II induced TK (the HSV-I enzyme produces the diphosphate). Cellular kinases complete the process to the triphosphate which inhibits the viral DNA polymerase to a greater extent than the host enzymes. Thus, BVDU is mostly incorporated in infected cells. Unfortunately, the agent is a better substrate for cellular thymidine phosphorylase than is thymidine and is thus degraded rapidly. Prolonged use has been linked with increased tumour development in laboratory animals. Arabinocytidine or cytarabine, 18 (araC), is not an effective agent as it inhibits cellular DNA synthesis to a greater extent than that of viral DNA [43]. Although triphosphorylated araC shows selectivity toward HSV DNA polymerase, this is offset by the lower concentrations of this species found in infected cells [44]. A series of 2'-fluorinated pyrimidine derivatives are more active and less cytotoxic. 2'-Fluoro-5-iodo-1-β-D-arabinofuranosylcytosine, 19 (FIAC), is active against DNA viruses especially HSV-I, II and herpes zoster [45, 46]. It is an excellent substrate to viral TK [47]. Some uracil analogues of FIAC (20, 21) are also active against these viruses. 1-β-D-Arabinofuranosylthymidine, 22 (araT), a naturally occurring nucleoside, is an excellent substrate to HSV thymidine kinase though it is also phosphorylated in healthy cells [48]. The triphosphate inhibits HSV DNA replication but may incorporate into cellular DNA [49, 50]. 5-Ethyl-arabinouridine (23) is nearly as active as araT against HSV-I and is less cyto-

toxic to uninfected cells [39].



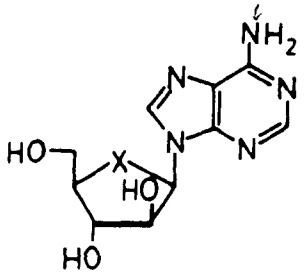
	X	Y	Z
11	I	H	OH
12	Br	H	OH
13	CF_3	H	OH
14	I	H	NH_2
15	C_2H_5	H	OH
16	C_3H_7	H	OH
17		H	OH
20	CH_3	F	OH
21	C_2H_5	F	OH
22	CH_3	OH	OH
23	C_2H_5	OH	OH



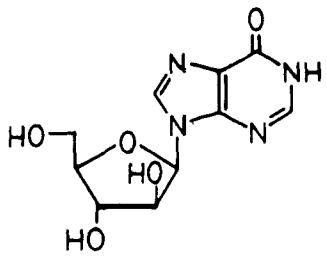
	X'	Y'
18	H	OH
19	I	F

Among the purine analogues, arabinoadenosine (araA) or vidarabine (**24**) has been found to be active against HSV-I, both in vitro and in vivo despite some cytotoxicity [51]. It is used for the treatment of herpes encephalitis, neonatal herpes and chicken pox. However, araA is relatively insoluble which hampers its administration and, when given systemically, it is rapidly converted to the less active hypoxanthine derivative (**25**) by the ubiquitous cellular enzyme, adenosine deaminase [22, 26]. The carbocyclic analogue of arabinoadenosine, cyclaradine (**26**), is active against

HSV-II and is resistant to adenine deaminase. (The properties of araA are further discussed in Chapter 2).



	X
24	O
26	CH_2

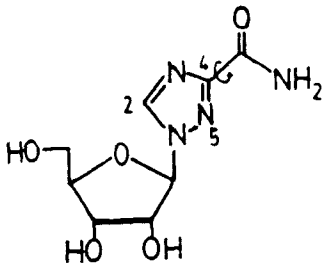


25

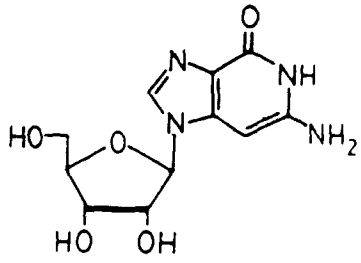
A number of nucleoside analogues bearing modified base residues also possess recognised antiviral properties, notably the 5-membered azaheterocycles [52]. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide or ribavirin (27) is a synthetic truncated nucleoside which possesses a broad spectrum of antiviral activity against both RNA and DNA viruses including the HSV pathogens [53, 54]. The mechanism for its action against RNA influenza A viruses may be explained in the following way. Rotation of the bond between C-4 and the amido group can cause inversion of the respective positions of the amine and keto groups. In one conformation, the amine function has the same orientation as that of adenosine and acts as a substrate for phosphorylation by cellular adenosine kinase. Cellular enzymes then produce triphosphorylated ribavirin. Depending on the orientation of the amine and keto group, this molecule can resemble either adenosine (NH_2-6) or guanosine (O-6) triphosphate and has been found to act as a competitive inhibitor for viral RNA polymerase in cell free assays. Because ribavirin is a ribonucleoside analogue, the risks of mutation due to incorporation into DNA should be minimal. In fact, ribavirin has not been found to incorporate into either viral or cellular DNA or RNA. This agent is thought to inhibit the viral-specific mRNA capping and methylating enzymes

28, 55. As a result, the mRNA is incorrectly transcribed causing either inhibition of viral protein synthesis or the production of faulty protein.

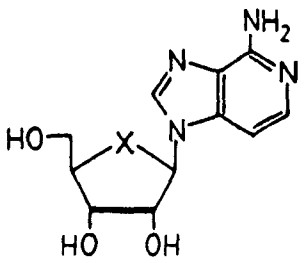
Base modified guanosine and adenosine nucleoside analoques have shown antiviral activity although these agents seem to carry a greater risk of carcinogenic potential. 3-Deazaguanosine (28) is effective against RNA viruses but exhibits cumulative toxicity [56]. 3-Deazaadenosine (29) has a broad spectrum of activity which, like that of ribavirin, has been attributed to interference with mRNA methylation processes essential to viral replication [57].



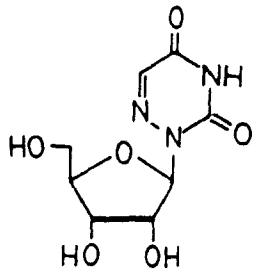
27



28



	X
29	O
32	CH_2

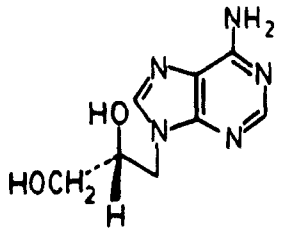


30

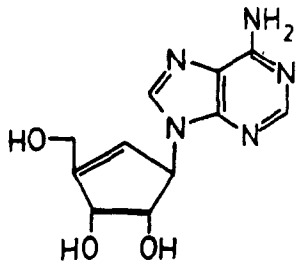
Several azapyrimidine nucleosides have a broad spectrum of activity in particular 6-azauridine (30) which inhibits both DNA and RNA viruses. However, its effect is weak on the HSV pathogens [25, 58] and its use is limited because of toxic side effects. Like other nucleoside analoques, this agent must first be phosphorylated in order to be effective. The

enzyme uridine kinase converts compound **30** to a nucleotide which then blocks DNA and RNA synthesis in both healthy and infected cells.

Certain analogues bearing modified sugar moieties also possess therapeutic value. The acyclic (S)-9-(2,3-dihydroxypropyl)-adenine, **31** (DHPA), and carbocyclic analogues 3-deazaaristeromycin (**32**) and neoplanocin A (**33**) are active against both DNA and RNA viruses [17, 57]. Their mode of action is thought to be similar to that of 3-deazaadenosine (**29**): (S)-DHPA inhibits (S-adenosyl-L-homocysteine)hydrolase which regulates transmethylation reactions essential to the production of mRNA whereas the (R)-isomer is inactive [59, 60]. This agent (**31**) also inhibits the action of adenosine deaminase and can thus be used to enhance the activity of araA (**24**) which is partially deactivated by this enzyme [25, 26].



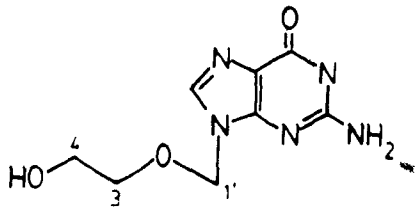
31



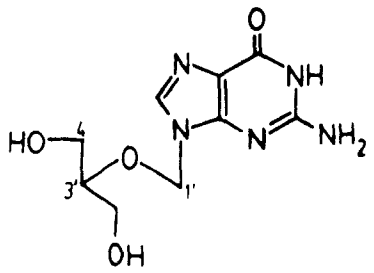
33

Acyclovir, **34** (ACV), an acyclo-nucleoside analoque of guanosine in which both the 2' and 3' sugar ring carbons are absent, was the first selective antiherpes drug reported for treatment of primary genital herpes [61, 62]. The molecule is active against both human and animal herpes viruses and is essentially non toxic to healthy cells. It is used both topically and orally in the treatment of HSV-I and II. ACV is an excellent substrate to viral TK and is converted to the monophosphate more quickly in infected than healthy cells. The agent is then diphosphorylated by the

host cell guanosine monophosphate kinase. Other cellular enzymes convert the di- to the triphosphate form which inhibits viral DNA polymerase to a greater extent than cellular polymerases. ACV acts as a chain terminator, its incorporation into viral DNA is self limiting due to the absence of a 3'-hydroxyl group. Analogues of ACV involving modifications of the guanine base are generally less active than the parent compound, as they do not bind as well to viral TK.



34

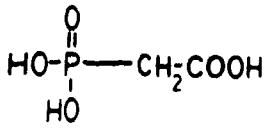


35

The activity of acyclovir has aroused interest in other nucleoside analogues of this type. The acyclic guanosine 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methylguanine or BIOLF-62 (35), possesses all the chemical functionalities of a deoxyribonucleoside but lacks the chiral, rigid furanose ring structure. Ogilvie and co-workers [63] first reported the antiherpes activity of this compound and this was subsequently reported by several other groups [21, 22, 64-67]. BIOLF-62 was found active against all viruses of the herpes group (HSV-I, HSV-II, cytomegalovirus, Epstein Barr virus) and against equine herpes virus both by plaque inhibition tests and animal studies. Patterns of mutant strain drug resistance (TK^+, DNA polymerase$^-$, ACV resistant) show that the mode of action of BIOLF-62 is different from that of other antiviral compounds [61, 68, 69]. It is phosphorylated more quickly than acyclovir, i.e. it is a better substrate to

virally induced TK. The triphosphorylated BIOLF-62 is present in greater concentration in infected than in non infected cells and inhibits viral DNA polymerase to a greater extent than the equivalent cellular enzymes. BIOLF-62 is a more selective viral DNA polymerase inhibitor than acyclovir and is easier to administer due to its greater solubility.

The combining of antiviral agents is often effective in treating infections. Two drugs implicated at different stages of the viral replication process can exert an action greater than the sum of their individual activities. BIOLF-62 exhibits such synergistic action with two other recognised antiherpes agents, phosphonoacetic acid, **36** (PAA), and phosphonoformic acid, **37** (PFA), in the treatment of HSV-I and HSV-II [70-72]. BIOLF-62 is also more effective against HSV-II when combined with Beta-interferon (an antiviral glycoprotein produced by virally induced leucocytes) [73].



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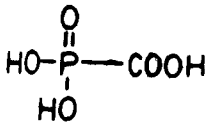


37

Only five antiviral drugs are approved for therapeutic use in the United States. These are the non-nucleosidic adamantamine (**6**), the base modified IDU (**11**) and TFT (**13**) as well as the sugar modified araA (**24**) and ACV (**34**). The prediction of the therapeutic value of antiviral agents is rendered difficult due to their complex mode of action, involving both cellular and viral enzymes. Nevertheless, in light of the activity of base modified nucleosides and the remarkable action of acyclic nucleoside

analogues, the combination of these two characteristics might prove of value. With the hope of duplicating or surpassing the therapeutic effectiveness of known drugs, a first objective proposed in this thesis is the synthesis of a series of derivatives, analogous to recognised acyclonucleoside antiviral agents. The coupling of a variety of heterocyclic-ring bases to the acyclic sugar-like segment of BIOLF-62 (**35**) was attempted and is described in the first chapter. The bases chosen to replace the purine ring of BIOLF-62 were selected on the basis of their known biological action or their resemblance to chemotherapeutic agents.

The Chemical Synthesis of Oligoribonucleotides

The recent advances in molecular biology, notably the cutting and handling of DNA fragments, have generated new applications for oligonucleotides of defined sequence. Because of remarkable improvements in the chemical approaches to nucleotide synthesis and the automation of these processes, synthetic oligonucleotides are now useful for DNA sequencing as well as directed mutagenesis. They also act as labelled probes to locate a specific gene and thus have potential for diagnostic applications [74]. Moreover, longer sequences are used for gene regulation and structure and function studies of nucleic acids.

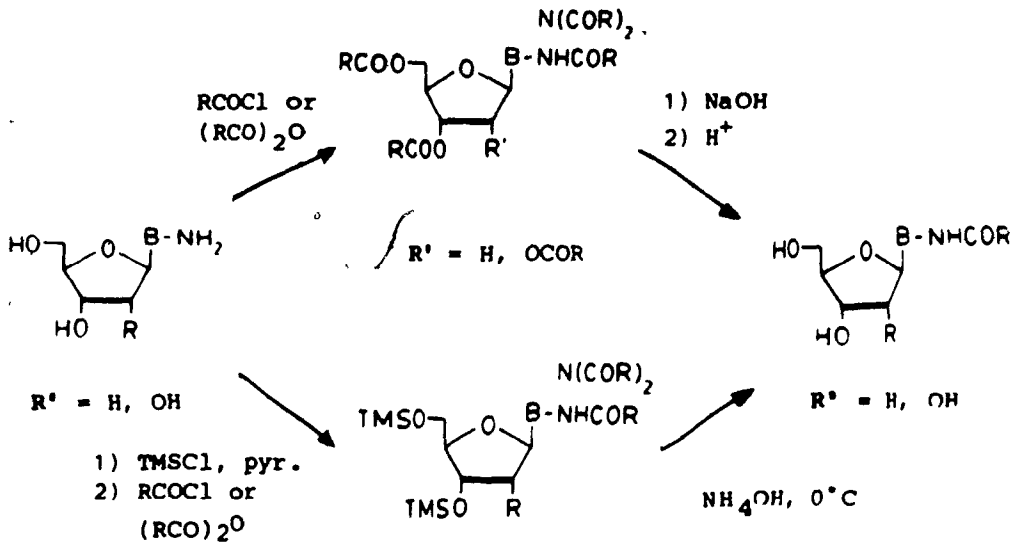
The synthesis of oligodeoxyribonucleotides involves a series of selective protection and deprotection steps of the nitrogenous base and the sugar moiety functional groups [75-77]. The common nucleic acid bases (Figure 1) possess amide or exocyclic amine groups susceptible to attack by nucleoside coupling reagents. These nucleophilic centers must often be derivatised to avoid possible side reactions which reduce yields and

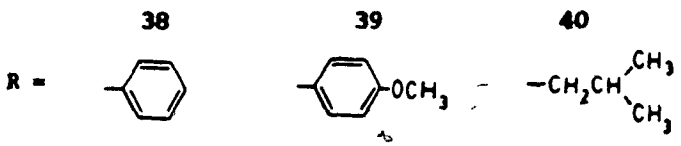
entail additional purification steps [77]. The deoxyribose sugar bears two potential reaction sites, the 3'- and 5'-hydroxyl groups (Figure 2). The condensation of two deoxyribonucleosides therefore requires selective protection of these functions to ensure the formation of the 3',5' nucleotide link (found in natural molecules) rather than 5',5' or 3',3' linkages. The presence of a third hydroxyl function in the 2' position of ribonucleosides renders the synthesis of oligoribonucleotides more complex than that of deoxyribonucleotides, and accounts for the slower development of RNA synthetic procedures. Selective protection of the 2'-hydroxyl group must also be undertaken to avoid the formation of undesired 2',5' linked ribonucleotides as well as the migration, under acidic conditions, of the phosphodiester moiety from the 3' to the 2' position [78]. The protecting groups used in these syntheses must be stable to the conditions required for internucleotide bond formation and must be introduced and removed under mild reaction conditions. Strong alkylating agents must be avoided as the nitrogen and oxygen base sites of these molecules, notably the N-7 position of purines, are particularly susceptible to electrophilic attack. Furthermore, while the phosphodiester backbone of DNA is stable to acid and base hydrolysis, the presence of the 2'-hydroxyl in RNA renders these molecules more susceptible to chain cleavage under both conditions via a 2',3'-cyclic phosphate [77]. It is evident from these properties that the selection of suitable protecting groups is crucial to the efficient synthesis of nucleotides.

The primary amine function of adenine, guanine and cytidine are generally protected as amides. The benzoyl (38), anisoyl (39) and isobutyryl (40) groups, used for A, C and G protection respectively, were initially introduced by Khorana et al.[79-81]. In addition to inhibiting

base residue modification during the coupling reactions, these protecting groups generally facilitate the handling of the afore mentioned nucleosides by increasing their solubility in organic solvents, thereby easing the purification of their derivatives. The amine protecting groups can be introduced by the original method described by Khorana and co-workers [82, 83]. Following peracylation of the nucleoside (G, A), the sugar acyl groups are selectively removed by basic hydrolysis (Scheme III). Peracylation is not required for protection of the cytidine amino group as it is basic enough for selective acylation by an anhydride. A more efficient method has lately been reported by Jones et al.[84]. This is a single step procedure in which the sugar hydroxyl groups are transiently persilylated prior

Scheme III





to acylation of the amine function (Scheme III). Unlike guanine and cytidine, adenine is diacetylated at N-6 by both procedures. The diacyl derivative of adenosine has been reported to be more stable to acid conditions than the monoacyl [85]. Nevertheless, the monoacyl is commonly used for nucleotide synthesis as one of the N-acyl groups is cleaved under the conditions used for hydrolysis of the acyl or silyl sugar hydroxyl protecting groups. At the end of nucleotide synthesis, the N-acyl groups are removed by treatment with concentrated ammonia.

Like the exocyclic amine groups, the O-6 amide position of guanine (2) can undergo side reactions (sulfonylation and phosphorylation) during oligonucleotide synthesis thus causing product degradation and lower yields [86, 87]. The carbobenzyloxy group (41) as well as a series of substituted ethyl groups [2-trimethylsilylethyl (42), 2-phenylthioethyl,

41	$COOCH_2Ph$	43	CH_2CH_2SPh, $CH_2CH_2SPhNO_2$	45	CH_2CH_2CN
42	$CH_2CH_2SiMe_3$	44	$CH_2CH_2PhNO_2$		

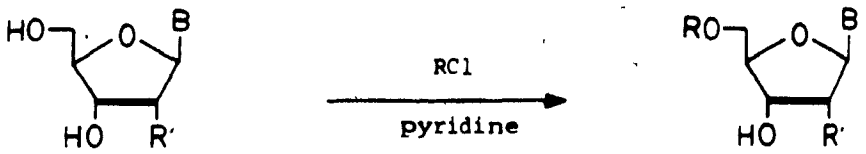
2-(4-nitrophenylthio)ethyl (43)] have been found suitable for O-6 protection [86-89]. Among the most commonly used are the nitrophenylethyl (NPE) (44) and the cyanoethyl (CE) (45) both of which can be removed by β-elimination reactions using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), tetrabutylammonium fluoride (TBAF) or ammonium hydroxide [86]. Although the CE group is relatively stable to triethylamine under anhydrous conditions, it is cleaved in aqueous solutions of this base [86].

The selection of 3' and 5' protecting functions which are easily introduced and selectively removed without affecting base protecting groups is imperative. As oligonucleotide synthesis must proceed by chain

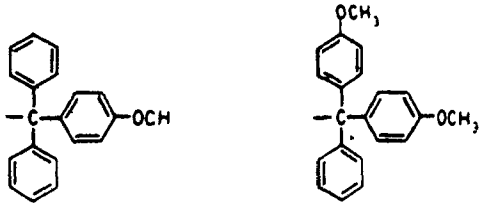
extension via either the 3'- or 5'-hydroxyl position, it is also advantageous to select two different hydroxyl protecting groups which can be removed independantly of one another. Synthetic strategies which proceed in the 5'→3' direction require transient protection of the 3'-hydroxyl group of each nucleoside. Conversely, 3'→5' directed syntheses require transient blocking of the 5'-hydroxyl groups and permanent protection of the 3'-hydroxyl group for the 3' terminal residue. Ribonucleotide synthesis presents the added problem of permanent 2'-hydroxyl protection for each residue. The formation of 3',5' linked ribonucleotides can be achieved by the same methods applied to DNA synthesis despite the hindrance at the 3' position caused by the presence of the 2'-hydroxyl protecting group.

The 5'-hydroxyl group of the deoxyribose and ribose moieties is usually protected with the monomethoxytrityl (MMT) or dimethoxytrityl (DMT) groups, both introduced to nucleotide synthesis by Khorana and coworkers [82, 83] (Scheme IV). These groups show good selectivity for reaction at the 5' position because of their bulk. In addition to conferring

Scheme IV



R = MMT DMT R' = H, OH

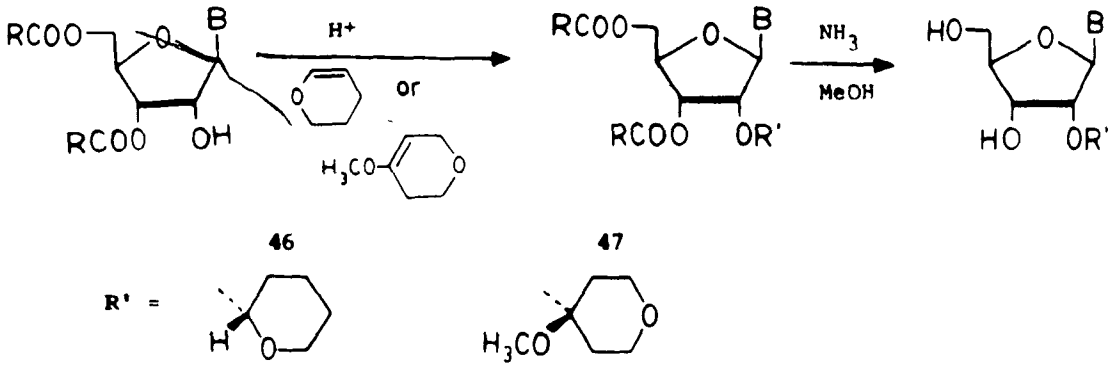


greater solubility in organic solvents, the trityl groups possess U V absorption properties which are useful in determining product yields. Both groups are easily cleaved by treatment with dilute acid so that a nucleotide chain can be extended from the 5'-terminal nucleoside. However, as the acid conditions used to remove the trityl groups can result in depurination of N-acetylated dA (and to a lesser extent dG), the DMT group, which is significantly more acid labile than MMT, is often preferred for DNA synthesis. In addition to the conditions initially reported for trityl group removal (80% CH_3COOH/H_2O) [90], several milder procedures have been proposed to minimise depurination. Among these, saturated zinc bromide solutions in CH_3NO_2 or CH_2Cl_2, dilute benzenesulfonic acid solutions in CH_2Cl_2/DMF or CH_2Cl_2/MeOH as well as dilute trichloroacetic acid (3%) have proved effective [91-94].

Several groups are commonly used to block the secondary hydroxyl functions of ribonucleosides as well as that of the 3' terminal deoxyribonucleosides. However, as both the 2'- and 3'-hydroxyls of ribonucleosides are secondary, attempted protection of the 2' position does not occur selectively and generally results in a mixture of isomers which are often difficult to separate. By first blocking the 3'- and 5'-hydroxyl positions, a protecting group can be introduced solely in the 2' position. The selective removal of the 3' and 5' groups thus leads to the desired 2' protected derivatives.

Acyl groups, used for blocking the exocyclic amine functions of the base residues, are also useful for the 3' protection of deoxyribonucleosides. However, acyl groups are not suitable for 2'-hydroxyl protection of ribonucleosides as they are particularly susceptible to isomerisation between the 2' and 3' positions under basic conditions [95]. The tetrahydro-

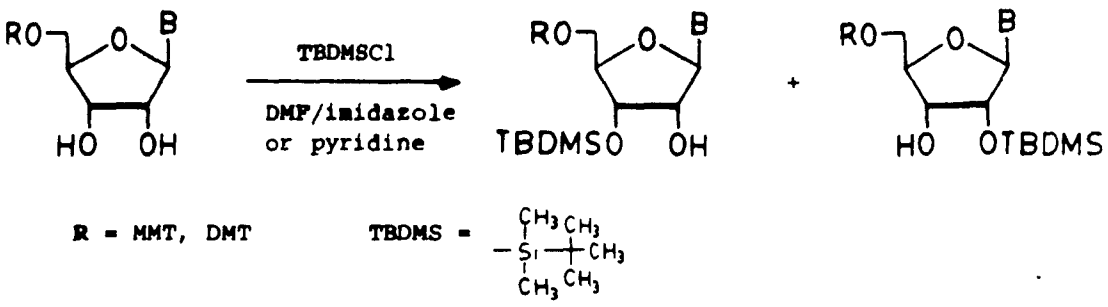
Scheme V



pyranyl group (THP) (**46**) has been used successfully for 2'-hydroxyl protection [96]. This group is easily introduced by treatment of a 5',3'-diacetylated ribonucleoside with 2,3-dihydropyran in the presence of acid (Scheme V) and can be removed under mildly acidic conditions [97]. Protection with a THP group introduces a chiral center. In order to avoid further complication of nucleotide chromatographic separation with the addition of each nucleoside, only one of the THP nucleoside isomers is selected for coupling. This disadvantage led Reese et al.[98] to introduce the symmetrical 4-methoxytetrahydropyranyl group (MTHP) (**47**) which leads to a single product. However, it was recently reported that the acidic conditions required to remove 5' protecting groups cause cleavage of 2'-THP groups to an extent unacceptable for nucleotide synthesis [99]. The MTHP group, which is more acid labile than the THP group, is also cleaved leading to nucleotide chain cleavage and isomerisation of phosphate linkages from the 3' to the 2' position [100].

The t-butyldimethylsilyl group (TBDMS), popularised by Corey and Venkateswarlu [101], was first applied to nucleoside protection by Ogilvie et al.[103, 104]. The TBDMS group is introduced by treatment of the 5' protected nucleoside with silyl chloride in the presence of a base (Scheme VI).

Scheme VI

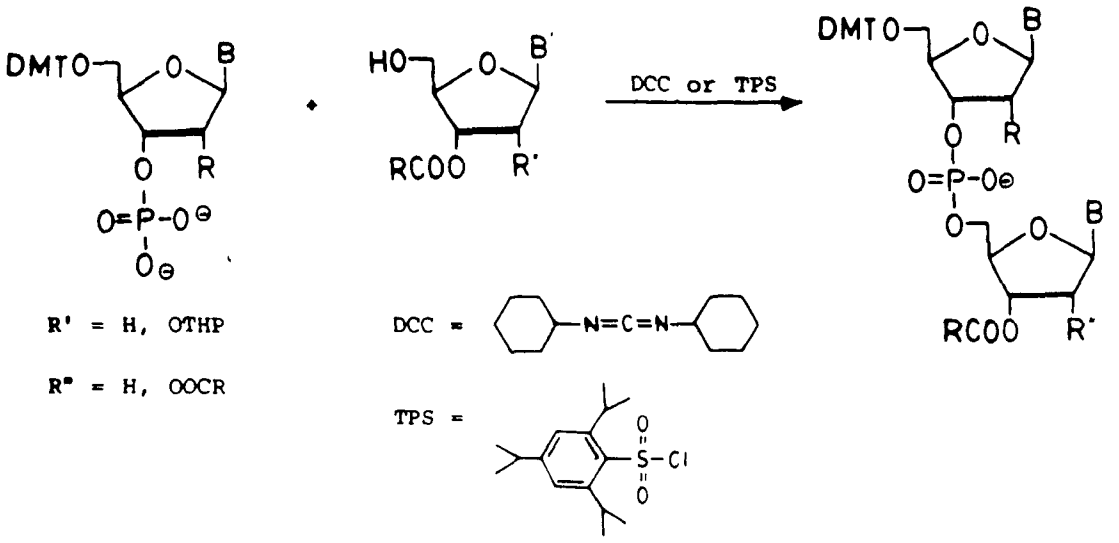


R = MMT, DMT TBDMS = $-Si(CH_3)_2C(CH_3)_3$

The 2'- and 3'-TBDMS isomers of 5' protected nucleosides are readily separable by chromatography; the preparation of the 2' protected product does not require previous blocking of the 3' position as in the case of the THP and MTHP derivatives. The TBDMS group in the 2' or 3' position is not cleaved under the acidic conditions required for removal of 5'-trityl groups. Although isomerisation between the 2' and 3' positions occurs in basic media [104], the TBDMS group is completely stable to nucleotide synthesis conditions. Furthermore, this group is removed under neutral conditions by treatment with tetrabutylammonium fluoride (TBAF).

The first joining of two deoxyribonucleosides with a natural 5'-3' internucleotide phosphate linkage was performed by the phosphate triester procedure (Michelson and Todd, 1955) [105]. The first syntheses of oligonucleotides, however, were achieved with the phosphodiester methodology introduced by Khorana [90] as well as Smrt and Sorm [96] (Scheme VII). In this procedure, a 5' protected mononucleotide is condensed with a 3' protected unit using dicyclohexylcarbodiimide (DCC) or triisopropylbenzenesulfonyl chloride (TPS) as condensing reagents. Following removal of the 5' trityl group, the procedure can be repeated to form short nucleotides which can then be linked together with enzymes (nucleotide kinase, DNA ligase) to form longer segments. Khorana and co-workers combined the chemical and

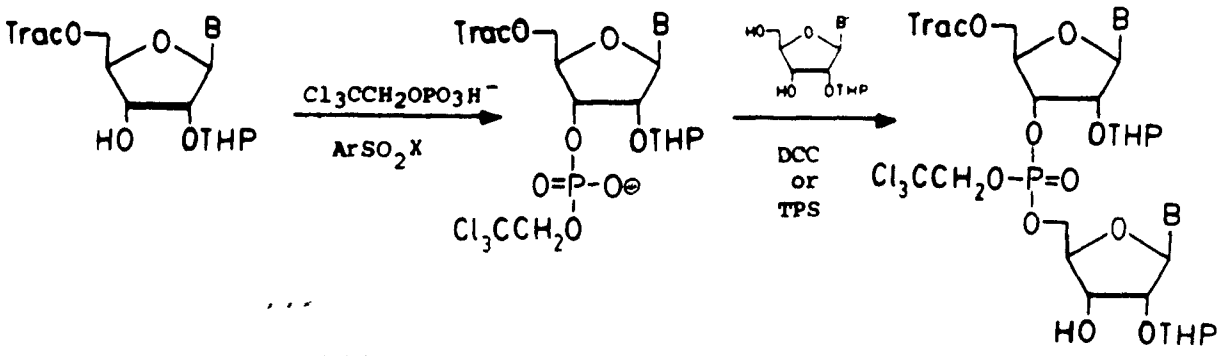
Scheme VII



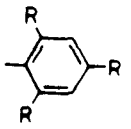
enzymatic techniques for the synthesis of the gene coding for the E.coli tyrosine suppressor tRNA [82]. However, the phosphate diester method suffers from poor yields and long reaction times and was not found effective for ribonucleotide synthesis [106]. Moreover, the nucleotide diesters formed are charged species and tend to undergo side reactions during subsequent condensations. The purification of intermediates is further complicated because the charged diesters exist as salts and are relatively insoluble in organic solvents. These problems become more difficult to overcome as chain length increases.

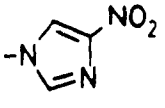
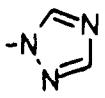
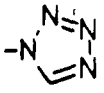
The chemical preparation of gene fragments was remarkably improved upon reintroduction of the triester methodology by Letsinger et al.[107, 108] and the development of supports for solid phase synthesis. The phosphotriester procedure was later adapted to the synthesis of ribonucleotides by Neilson [109, 110] (Scheme VIII) and subsequently employed by other groups [111, 112]. Neilson found that protection of the 3'-hydroxyl group is not necessary as no 3'-3' dimer formation was observed. This position is

Scheme VIII



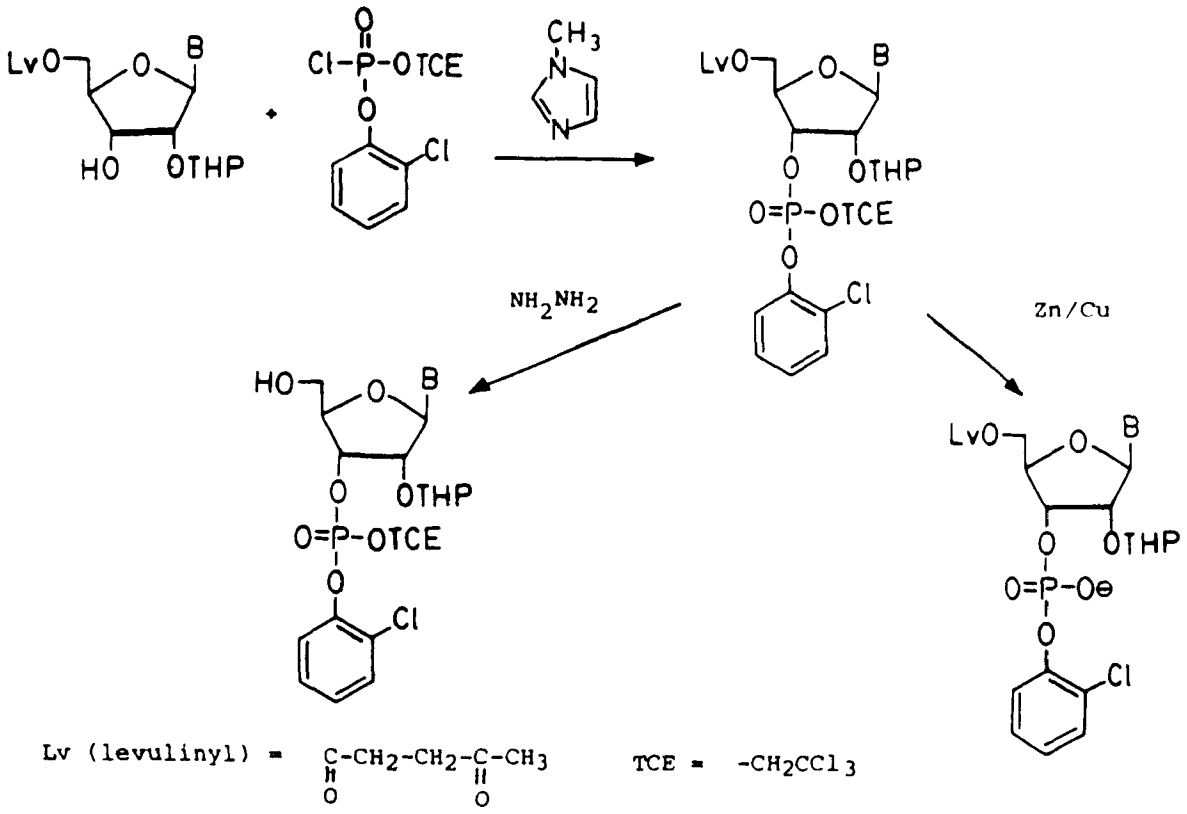
Trac = $OCCH_2OC(Ph)_3$

Ar =  R = CH_3, $CH(CH_3)_2$)

X = Cl,  ,  ,  .

considerably less accessible than the 5'-hydroxyl due to steric hindrance caused by the 2' protecting group. Unlike the diester approach, the phosphate triester procedure leads to formation of uncharged, fully protected phosphates which are amenable to chromatographic separation and compatible with hydrophobic supports. At the end of nucleotide synthesis, prior to removal of the sugar and base protecting groups, the phosphate protecting groups are cleaved by treatment with a mild nucleophile. However, phosphotriesters are considerably less stable to hydrolysis than diesters and the risk of chain cleavage is greater. The greater sensitivity of triesters must be considered when selecting conditions for the protection and deprotection of the sugar hydroxyl groups and in determining the order in which the various operations are performed. Several refinements to the triester approach have been developed notably the modified triester approach, introduced by Cramer [113] and Narang [114], which was adapted to ribonucleotide synthesis by Van Boom et al.[115] (Scheme IX). This method allows the

Scheme IX



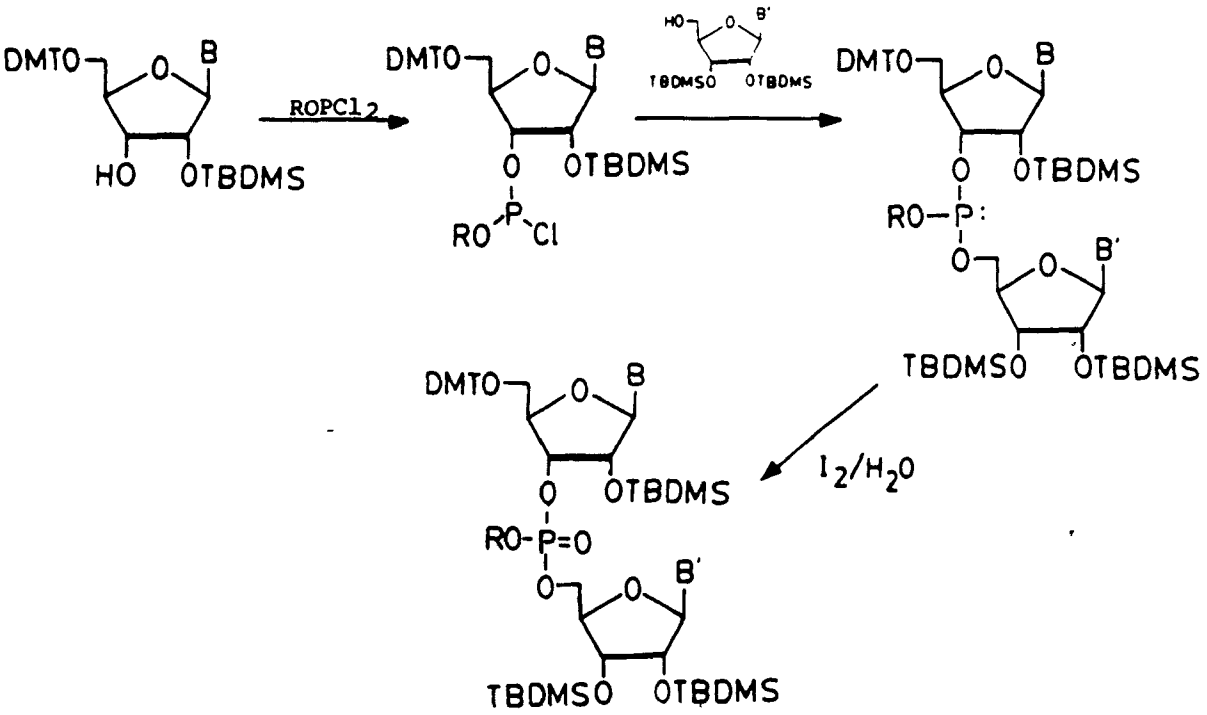
Lv (levulinyl) = $\overset{O}{\overset{\|}{C}}-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-CH_3$ TCE = $-CH_2CCl_3$

preparation of fully protected nucleoside 3'-phosphates which are easily purified and are stable enough for storage [113, 114]. The phosphate is prepared by treating the 2',5' protected nucleoside with a phosphochloridate. By selecting appropriate 5'-hydroxyl and phosphate protecting groups it is possible to remove them independantly for coupling in either the 5' or 3' position. As in the phosphodiester procedure, phosphotriester approaches require the use of condensing reagents such as DCC, TPS or a variety of arylsulfonolides of heterocyclic bases (imidazole, triazole and tetrazole). The principal drawback of the phosphate triester procedures is the tendency of the arylsulfonyl activating reagents to sulfonylate the unprotected amine and amide functions of the bases as well as the free 5'-hydroxyl group of nucleosides [116]. The competition between phosphorylation and sulfonylation at this position leads to reduced yields and complicated

purifications due to side product formation [117, 118].

The triester methodology was further improved with the introduction of the phosphodichloridite or phosphite triester procedure by Letsinger et al.[119, 120]. This approach involves the phosphorylation of nucleosides without the involvement of arylsulfonyl coupling reagents and leads to condensation yields superior to those obtained by previous methodologies. The dichloridite procedure has also been applied to the synthesis of ribonucleotides [121, 122]. Ogilvie et al.[123] combined this procedure with the use of the TBDMS group for 2'-hydroxyl protection (Scheme X). The 3'-hydroxyl of the first nucleoside is treated with a phosphodichloridite in the presence of a base followed by reaction with the 5'-hydroxyl of a second nucleoside. Due to the reactive nature of the dichloridite, conden-

Scheme X

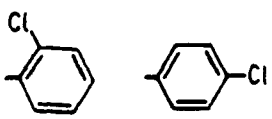


R = CH_3, CH_2CCl_3, CH_2CH_2CN

sation reactions are very rapid and, consequently, are performed at low temperature (-78°C) to minimise formation of a 3',3' linked dimer. The phosphite triester produced (P^{III}) is then oxidised to the more stable phosphate (P^{V}) by treatment with iodine and water. As in the phosphate triester procedure, the molecules produced are neutral until the phosphate protecting groups are removed, at the end of the synthesis.

The functional groups used for phosphate protection in the triester procedures must remain intact until the end of nucleotide synthesis. These groups must therefore be stable to the conditions used for cleavage of the hydroxyl protecting groups. Furthermore, the conditions needed to remove phosphate protecting groups must not lead to chain cleavage or isomerisation of the phosphodiester linkages. Some groups are removed by attack of a nucleophile directly at the phosphorous center resulting in O-phosphonyl cleavage. The removal of phenyl protecting groups (**48**), used by Reese *et al.*[124, 125], requires treatment with a hydroxyl ion and is accompanied by some internucleotide cleavage [126]. This problem prompted the use of other groups, such as the chlorophenyl (**49**) [121, 128] and methyl (**50**) [130, 131], which are removed by treatment with mild nucleophiles (aldoximate and thiophenate anions, respectively). However, some methylation of the amide function (N-3) of thymidine residues has been reported to occur during oligonucleotide synthesis when the methyl protecting group is used [129]. Other phosphate protecting groups are removed by specific O-alkyl cleavage and do not involve attack of a nucleophile at the phosphorous atom. These

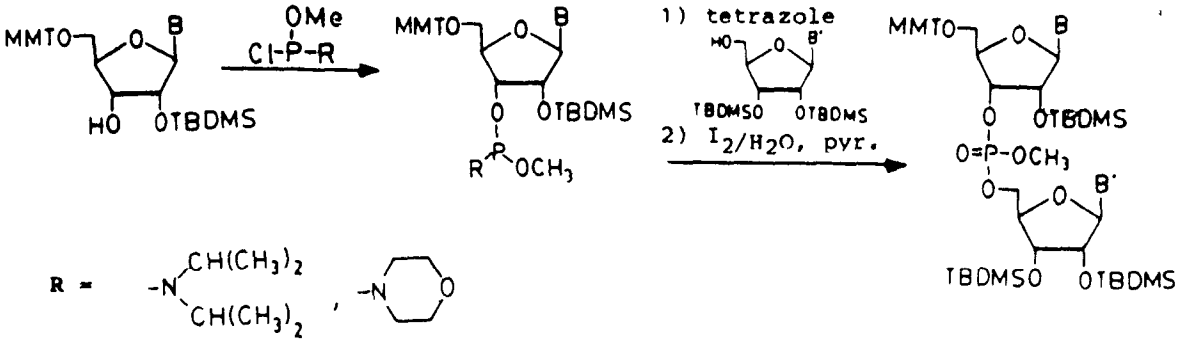
48 CH_2Ph **50** CH_3 **51** CH_2CCl_3

49  **52** $C(CH_3)_2CCl_3$

include the 2,2,2-trichloroethyl (TCE) (51), the cyanoethyl (CE) (45), nitrophenylethyl (NPE) (44) and the 1,1-dimethyl-2,2,2-trichloroethyl (52) functions. The TCE group [130], which was used in the original phosphite triester work [119, 120], is stable to both acid and mild base and is cleaved by treatment with Zn/Cu couple in DMF [130]. However, the TCE removal procedure has some drawbacks. The reaction requires heating and yields range between 20 and 90%. Moreover, reaction times vary from 2 to 24 hours. The success of the deprotection is dependant on the quality of the Zn/Cu couple which must be freshly prepared. The CE and NPE groups are also used for the O-6 protection of guanosine as discussed earlier. The CE group (45) [131], used in the first phosphate triester syntheses [107, 108], has been found fully compatible with the dichlorophosphite methodology [132]. Furthermore, the CE group is cleaved without side reactions upon treatment with triethylamine in acetonitrile (40%) or with concentrated aqueous ammonia. The NPE group (44) [133] and the recently introduced dimethyltrichloroethyl group (52) are not widely used for phosphate protection. Both 44 and 52 are cleaved under mild conditions i.e. by treatment with DBU [134] and sodium naphtalenide in hexamethylphosphoramide (HMPA) [135], respectively.

Limitations of the phosphodichloridite method include the difficulty in handling chlorophosphites due to their moisture sensitivity and the possible formation of 3',3' linked nucleotides. These have led to the development by Caruthers et al. of an improved procedure using the nucleoside morpholino- or diisopropylaminophosphoramidites [136-138]. This approach (Scheme XI) has also been used by other groups for the synthesis of deoxyribonucleotides [139, 140] and has been applied to the preparation of ribonucleotides both in solution [141] and on solid support [142]. Phos-

Scheme XI



phoramidites, easily prepared in high yield, are relatively stable to both oxidation and hydrolysis. At the coupling stage, the amidite is activated by an acid catalyst, usually tetrazole. The second nucleoside is then added to the reactive tetrazolide thus formed.

The synthesis of oligonucleotides has become considerably more efficient with the development of solid supports, which eliminate the need for isolation and purification of the product after each nucleoside coupling. In automated systems, the support is packed in a column through which reagent solutions are successively pumped. Unreacted reagents and their products as well as any 3',3' linked dimer which might have been produced can thus be easily washed off. Moreover, it is possible, by employing an excess of reagents, to drive coupling reactions to near quantitative yields.

Upon completion of the synthesis, the oligonucleotide chain must be deprotected. The usual sequence first involves removal of the phosphate protecting groups followed by displacement of the exocyclic amine protecting groups and, in the case of solid phases syntheses, cleavage of the nucleotide from the support. A principal advantage of using the cyanoethyl group for phosphorus protection, is that all the preceding operations can

be performed in a single step by treatment with ammonium hydroxide. Removal of the 2' protecting group follows. When the nucleotide is to be isolated by electrophoresis, removal of the trityl group of the 5' terminal nucleoside may precede all other deprotection steps. Alternatively, detritylation can be performed last, following HPLC purification of the partially deprotected nucleotide.

As has been indicated, the efficient synthesis of oligonucleotides depends upon the selection of suitable protecting groups for the phosphorous as well as several of the nucleoside functional groups. The most effective synthetic procedures for the preparation of ribonucleotides have been shown to be the phosphite triester [143] and, more recently, the phosphoramidite methods [142]. The compatibility of the 5'-trityl and 2'-TBDMS hydroxyl protecting groups with these procedures as well as the ease of preparation and purification of the correspondingly derivatised ribonucleosides have also been demonstrated. Thus, these groups are indicated for use with nucleosides possessing sugar rings other than ribose. One objective of this work, presented in Chapter 2, was the preparation and full characterisation of several such derivatives of the antiviral agent arabinoadenosine. These dimethoxytritylated and silylated compounds are of interest for their eventual incorporation into a nucleotide chain as a possible means of enhancing the biological activity of arabinoadenosine. ^{1}H and ^{13}C NMR studies of nucleosides, nucleotides and their derivatives have shown the value of NMR in the characterisation of these molecules. A further objective was to report the application of ^{29}Si INEPT NMR to the characterisation of some of the silylated nucleosides widely used as intermediates in ribonucleotide synthesis. This is discussed in Chapter 3. The search for hydroxyl protecting groups suited to nucleotide synthesis,

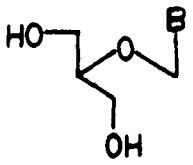
particularly those introduced and removed under neutral conditions, is a non ending one. In conclusion, a final aim of the present work was to discuss the potential for the use of 2,4-dinitrobenzenesulfenyl as a hydroxyl protecting group for the synthesis of ribonucleotides and the extent of its compatibility with the phosphodichloridite coupling procedure.

CHAPTER ONE

Preparation of Novel Heterocyclic-Ring Analogues of BIOLF-62

1.0 Introduction

The method used in the synthesis of analogues **68**, **70**, **72**, **74**, **81**, **88** and **90** was modeled on the procedures developed in this laboratory for the preparation of acyclic purine and pyrimidine nucleoside analogues [144]. The heterocyclic bases were selected on the basis of their previously reported antiviral activity or their resemblance to known drugs.



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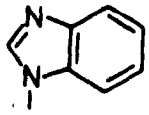
68



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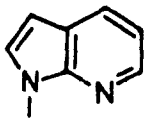

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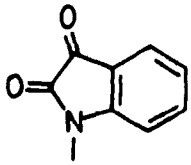

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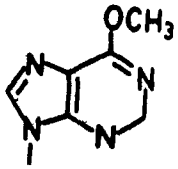

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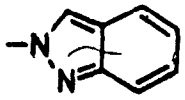

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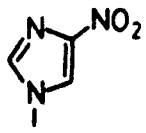
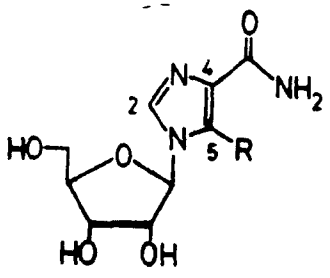
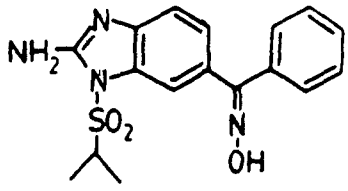


90

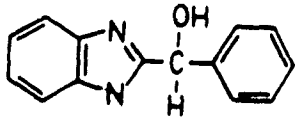
The imidazole, 4-nitroimidazole and benzimidazole bases were selected for coupling to the acyclic moiety of BIOLF-62 (35) because of their similarity to known biologically active agents. The antiviral activity of ribavirin (27) has led to interest in triazole, tetrazole and imidazole nucleoside derivatives and the structural requirements for their therapeutic effectiveness. The activity of such analogues is related to their stability in the anti conformation around the glycosidic bond (N-1,C-1'). This conformation is required for phosphorylation by adenosine kinase. 1-β-D-Ribofuranosylimidazole-4-carboxamide and its 5-fluoro derivative (53, 54), represented in the anti conformation, are the most promising such derivatives to date [145]. Although these compounds are less potent than ribavirin, they are active against a greater variety of pathogens. Other studies have confirmed that an alkyl substituent on C-2 or C-5 diminishes the activity of imidazole ribonucleosides [146, 147]. Benzimidazole derivatives are also noted for broad spectrum antiviral activity, examples of which are compounds 55 and 56. They exhibit reasonably low toxicity, are well absorbed when administered orally and are thought to act either by blocking viral penetration or inhibiting the virus directed syntheses [25, 148, 149].



	R
53	H
54	F



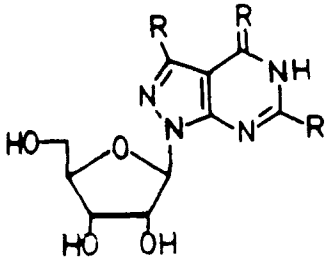
55



56

As previously noted, deazapurine analogues (28, 29) have exhibited antiviral action by interfering with methylation reactions involving mRNA.

In addition, pyrazolo[3,4-d]pyrimidine nucleosides of the general structure 57 have shown antitumour and antiviral activity particularly against the herpes group viruses [150, 151]. Such compounds are remarkably stable to enzymatic hydrolysis of the glycosidic linkage [152]. Several bicyclic nitrogenous bases similar to those of the above nucleosides were also selected. The preparation of the isatin analogue was of interest as thiosemicarbazone derivatives of isatin are recognised for their activity against DNA viruses, particularly the pox viruses. Isatin-3-thiosemicarbazone (58) and 1-methylisatin-3-thiosemicarbazone (59) interfere with the synthesis of viral structural proteins and cause the formation of immature viruses [16, 22, 25]. Recently a cyclopropane derivative of isatin (60) has been found to be significantly active against influenza viruses thus showing that the thiosemicarbazide moiety is not essential to activity [153]. The replacement of the cyclopropylmethyl moiety with the acyclic portion of BIOLF-62 (35) might extend the activity of this base to the DNA herpes viruses.



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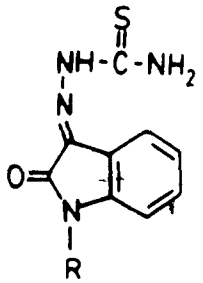
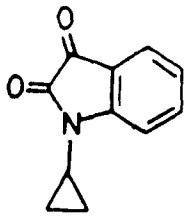


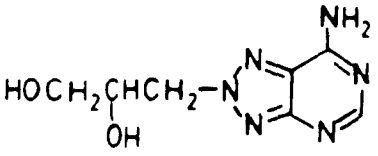
	R
58	H
59	CH_3



60

The preparation of two acyclic analogues of 8-azaadenosine has been reported (61, 62) but these compounds do not possess anti-herpes activity [154]. However, the arabino derivative of 8-azaadenine is effective <u>in vitro</u> against HSV-I and II [155]. The coupling of this base with the acyclic

moiety of BIOLF-62 was of interest as the resulting analogue might possess significant antiviral activity. In addition, this analogue might be resistant to adenine deaminase which converts 8-azaadenosine to its hypoxanthine derivative, 8-azainosine (**63**), a compound known to be toxic to mammalian cells [156].



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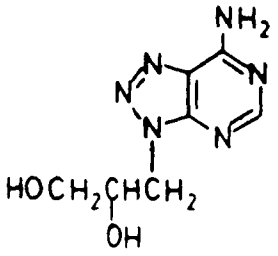
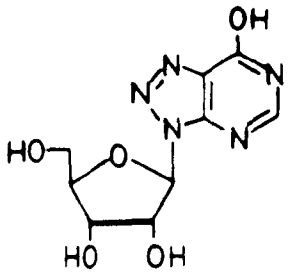


62



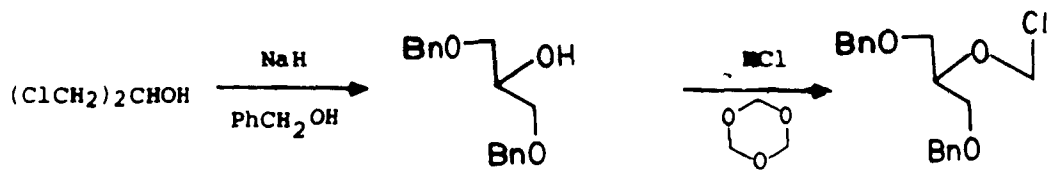
63

1.1 Synthesis of the Analogues

The first step undertaken was the synthesis of the acyclic alkyl chloride, **66** (Scheme XII), to be later coupled to the various nitrogenous bases. 1,3-Dibenzyloxy-2-propanol (**65**) was prepared in 97% yield by the reaction of 1,3-dichloropropanol (**64**) with sodium benzylate under argon atmosphere. Compound **65** was then treated with paraformaldehyde in anhydrous 1,2-dichloroethane while a stream of gaseous hydrochloric acid was bubbled through the solution for three hours. The water produced in the reaction was eliminated by absorption onto calcium chloride ($CaCl_2$) and precautions were taken in the subsequent handling of the moisture-sensitive 1,3-dibenzyloxy-2-chloromethoxypropane (**66**). The chloromethyl derivative was thus obtained as a mixture with compound **65** in 86% yield (deter-

mined by ^1H NMR) and was used without further purification in the preparation of the protected acyclic analogues.

Scheme XII



64 **65** **66**

A summary of the preparation conditions, yields and physical properties of the analogues prepared is presented in Table II.

TABLE II

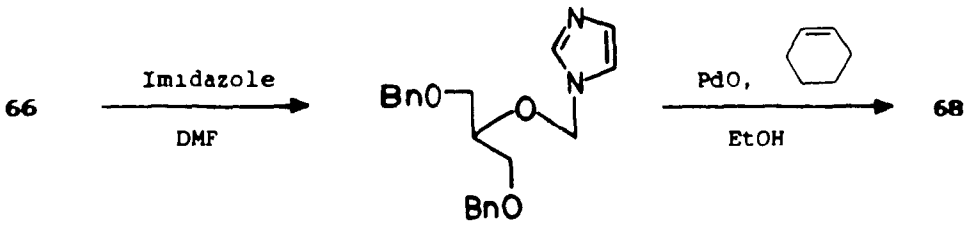
Preparation and Physical Properties of the Acyclic Analogues[1]

Product	Coupling Conditions	Yield (%)	Debenzylation Conditions	Yield (%)	Melting Point (°C)	λ_{max} nm (EtOH)	λ_{min} nm (EtOH)	R_f	
68	DMF	67	PdO/C_6H_{10}	86	38-40	210	200	0.48	a
70	DMF	83	PdO/C_6H_{10}	66	111-112	280	277	0.35	b
	HMDS/TBAI	60				274	222		
	$(Bu_3Sn)_2O$	67				245			
72	TEA/DMF	60	BCl_3/CH_2Cl_2	64	68-70	287	242	0.53	c
	TEA/THF	93							
74	TEA/THF	88	BCl_3/CH_2Cl_2	76	107-108	297	270	0.41	d
			PdO/C_6H_{10}	35		243	218		
81	TEA/DMF	56	BCl_3/CH_2Cl_2	89	132-133[2]	247	223	0.47	e
	HMDS/I_2*	65							
88	TEA/THF	94	BCl_3/CH_2Cl_2	89	---	293	283	0.27	d
						275	235		
90	TEA/THF	95	BCl_3/CH_2Cl_2	74	113-114	285	247	0.46	c

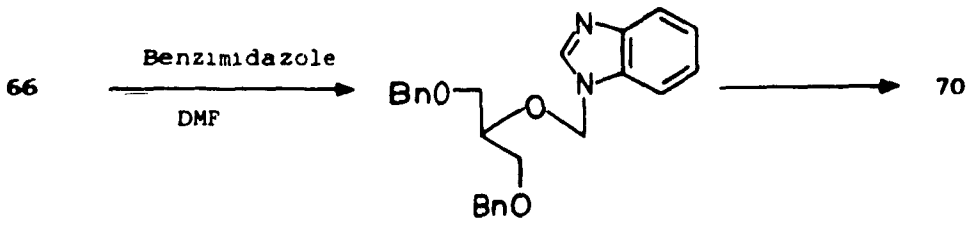
1) Coupling reactions performed with (**66**) except (*) performed with (**84**).
2) Literature melting point: 135-136°C (ref.161). TLC solvents: a) CH2Cl2/MeOH (7:3); b) CH2Cl2/EtOH (4:1); c) EtOAc/MeOH (9:1); d) CH2Cl2/EtOH (9:1); e) EtOAc/CHCl3/EtOH (5:4:1).

1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]imidazole (68) was prepared by coupling a threefold excess of imidazole to the chloromethyl ether (66) in anhydrous DMF (Scheme XIII). The reaction mixture was stirred overnight at room temperature to yield 67% of 1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]imidazole (67). The benzyl protecting groups were removed by a catalytic transfer hydrogenation procedure in which a solution of 67 in absolute ethanol was refluxed for 2 hours with palladium oxide using cyclohexene as the hydrogen donor. The imidazole

Scheme XIII



67



69

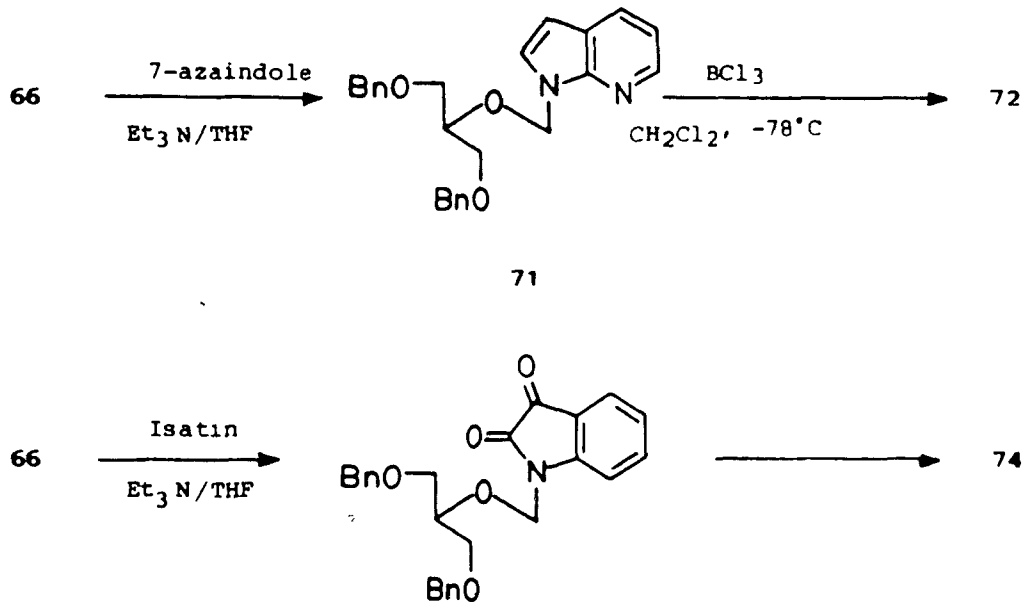
analogue (68) was obtained in 86% yield as a yellow oil which crystallised slowly from methanol/water. The product exhibits a UV absorption maximum consistent with the value reported for 1-methylimidazole λ_{max} (MeOH) = 211 nm) [158]. The elemental analysis results confirm the composition of 68 if half a mole of water is included. This problem is a recurring one as

the two hydroxyl groups present in the acyclic moiety render difficult the complete elimination of water introduced during crystallisation. ^{1}H and ^{13}C NMR characterisation of this and the following analogues is discussed in section 1.2.

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]benzimidazole (69) was prepared in 83% yield by the coupling of benzimidazole with the chloromethyl ether (66) in the same manner as above with an initial two hour cooling period at 5°C for two hours. Alkylation products other than the desired isomer were not apparent by TLC. The benzyl groups were removed by catalytic transfer hydrogenation to yield the desired derivative (70) in 66% yield. White crystals were obtained from $MeOH/H_2O$. The structure of 70 was confirmed by elemental analysis. However, electron impact mass spectrometry did not help in its characterisation, a molecular ion peak was not detected; the highest mass peak observed was [benzimidazole +] at m/z: 118. Similar results were obtained for other acyclic analogues subjected to EI mass spectrometry and this technique was abandoned because of its limitations for the characterisation of such compounds.

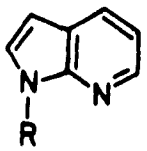
The preparation of 1-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-7-azaindole (71) was then undertaken (Scheme XIV). The original coupling procedure employed was modified from those reported by Ogilvie and coworkers 35, 36. The chloromethyl ether (66) was added to a cooled solution (5°C) of 7-azaindole in anhydrous DMF. Triethylamine was used as a scavenger for the hydrochloric acic produced. The reaction was stirred two hours at 5°C then allowed to warm to room temperature and stirred another 12 hours. The protected derivative (71) was isolated as a yellow oil in 50% yield. When the reaction was repeated without the initial cooling period,

Scheme XIV



the yield was improved to 60% and no side products were observed. However, when anhydrous THF was used, the recovered yield increased to 93% despite this solvent's lesser polarity and the diminished solubility of 7-azaindole. The moisture content of the DMF employed was determined to be approximately 20 ppm by Karl Fisher titration thus eliminating the presence of water as a cause for the lower yields previously obtained. As both solvents are dipolar aprotic, the yield differences are in all likelyhood attributable to the more facile solvent elimination and subsequent column purification of the THF reaction relative to that in DMF. The debenzylation of (71) was attempted by catalytic transfer hydrogenation (PdO/cyclohexene in refluxing EtOH). The reaction, monitored by TLC, progressed as expected with disappearance of the starting material. A slower moving compound was observed and there also appeared to be a considerable amount

of a UV absorbing material which remained at the TLC base line. These materials were isolated but could not be identified by ^1H NMR. The characteristic methylene signal of the pseudo-glycosidic bond (δ = 5-6 ppm) was not apparent and although signals in the aromatic region corresponding to the heterocyclic base protons were present, neither compound was identical to the 7-azaindole used as starting material. A second catalytic transfer hydrogenation was attempted using ammonium formate (6 eq.) as the hydrogen donor and palladium on carbon as the heterogeneous catalyst. The reaction was performed in DMF at room temperature according to a procedure developed for peptide synthesis by Anwer and Spatola [159]. Although the reaction time reported for the removal of a benzyl ether group is 10 minutes, TLC monitoring revealed that the protected 7-azaindole analogue (71) failed to react even after 90 minutes. Still no change occurred when the reaction was heated to 45°C overnight. As the debenzylation is reported to proceed effectively in DMF or methanol [159], the reaction was repeated at room temperature substituting methanol for DMF. The starting material disappeared rapidly and a new product was formed. Isolation and ^1H NMR characterisation revealed this to be 1-hydroxymethyl-7-azaindole (75). The structure was confirmed by electron impact mass spectrometry which showed a molecular ion (m/z: 148) and a base peak (m/z: 118 [7-azaindole$^+$]).



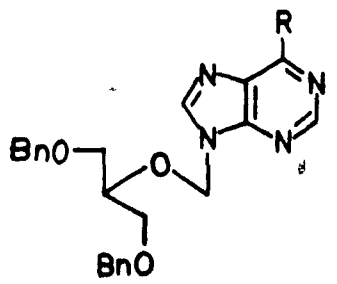
	R
75	$-CH_2OH$
76	$-CH_2OCH_3$

The debenzylation of 71 was then attempted by treatment with boron trichloride (8 eq.) in anhydrous dichloromethane for two hours according to a procedure developed by Seela and Menkhoff [160]. The reaction was per-

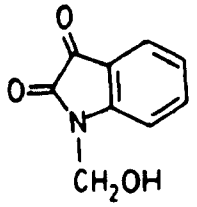
formed at low temperature (-78°C) to minimise cleavage of the pseudo-glycosidic bond. In a first attempt, the reaction was carried out at high concentration (10 mL of dichloromethane per mmol. of 71) and was quenched with methanol at room temperature. These conditions resulted in cleavage of the ether bond in the alkyl residue yielding 1-hydroxymethyl-7-azaindole (75). A minor product, faster moving by TLC, was identified as methoxymethyl-7-azaindole (76) by ^{1}H NMR but this could not be confirmed by mass spectrometry as the highest mass peak apparent was for m/z· 148 [M$^+$ - CH_2·]. The reaction was repeated with an increased dilution factor (70 mL/mmol. of 71) and the quenching with methanol as well as the neutralisation with triethylamine were carefully performed at low temperature. The desired 1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-7-azaindole (72) was recovered in 64% yield. The oil obtained was easily crystallised from methanol to form white flakes. NMR characterisation, discussed in section 1.2.2, and elemental analysis (section 5.1.9) confirmed the product structure and composition.

1-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-isatin (73) was prepared in the same manner as 71 (Schème XIV). The reaction, performed at room temperature, afforded 73 in 70% yield due to difficulties encountered in the purification of this derivative. The yield was subsequently improved to 88% by repeating the procedure with moderate heating (40°C). Initial attempts at removing the benzyl groups from 73 by catalytic transfer hydrogenation (palladium oxide/cyclohexene) in refluxing absolute ethanol proved unsatisfactory. The slow removal of a single benzyl group was apparent by TLC monitoring. The starting material was eventually consumed upon the addition of more PdO and cyclohexene giving rise to the desired deprotected analogue (74) in 35% yield as well as several uniden-

tified lesser products. Similar problems were reported by Gillen [161] for the catalytic transfer hydrogenation debenzylation of the acyclic adenosine analogue (77). It was found that the removal of a first benzyl group was difficult but the reaction subsequently proceeded smoothly and cleanly to yield the dihydroxyl derivative. The initial difficulty was attributed to a possible stacking interaction between the purine ring and the benzyl groups rendering them inaccessible to the catalyst. The treatment of 73 with boron trichloride at high concentration resulted, as in the case of the 7-azaindole analogue, in the formation of a 1-hydroxymethyl derivative (78) characterised by ^{1}H NMR and UV spectroscopy. Absorption maxima at 300 and 243 nm were observed in the UV spectra and these values are consistent with those reported for 1-methylisatin ($\lambda_{max(EtOH)}$ = 304, 424 nm) [158]. When the reaction was repeated at higher dilution, the desired analogue 74 was recovered in 76% yield as a waxy solid which crystallised to bright orange needles in diethyl ether. The structure was confirmed by elemental analysis and NMR spectroscopy.



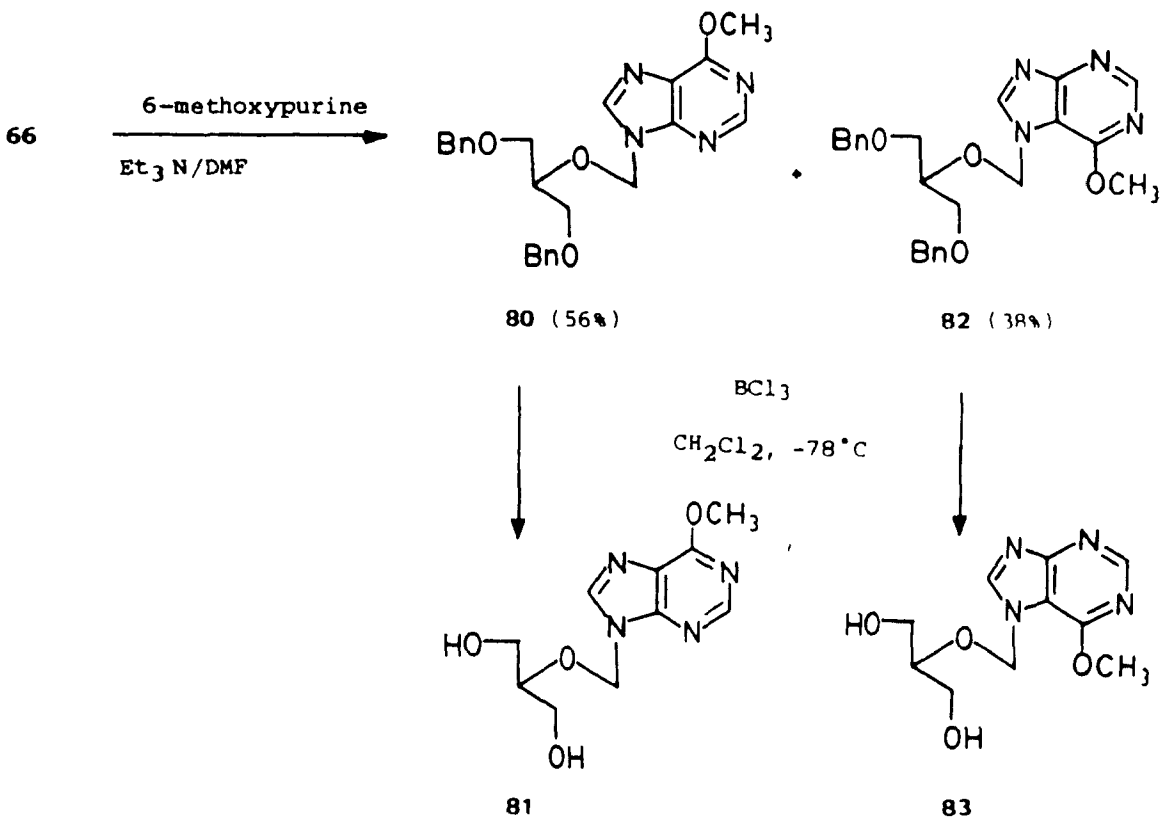
	R
77	NH_2
79	Cl



78

The preparation of 9-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-6-methoxypurine (80) had been published by Ogilvie et al.[157]. In the reported procedure, the previously described 6-chloropurine analogue (79) [147] was converted to 80 in 56% yield, by alkaline displacement (CH_3ONa, MeOH). In the course of the work undertaken here, it was found that this

Scheme XV

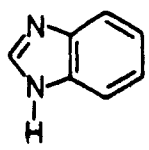


analogue (**80**) can be prepared by direct coupling of 6-methoxypurine with the chloromethyl ether (**66**) in DMF using triethylamine as a base (Scheme XV). The reaction was stirred 12 hours at room temperature to give the desired 9-isomer (56% yield) and the less favoured 7-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-6-methoxypurine (**82**) (38% yield). The two isomers are easily differentiated by their ^{1}H and ^{13}C NMR spectra, discussed in section 1.2.3, and by their UV maxima at 247 nm for **80** and 258 and 232 nm for **82**. The absorption values of the desired deprotected analogue (**81**) (λ_{max} = 247 nm, λ_{min} = 223 nm) and those of the deprotected 7-isomer (**83**) (λ_{max} = 256, 230 nm; λ_{min} = 247, 222 nm) are consistent with the trend for higher wavelength maximal absorption values reported for N-7 versus N-9 substituted purines [162]. The treatment of **80** with boron tri-

chloride in anhydrous dichloromethane (-78°C) afforded the desired 6-methoxypurine analogue (**81**) (89% yield). The yellow oil obtained was easily crystallised from methanol to a white powder. Elemental analysis results were unsatisfactory due to the large quantities of solvent present in the sample even after grinding and drying under reduced pressure at 68°C for several days. This was confirmed by ^{1}H NMR which showed peaks due to methanol and water. Peak integrations were consistent with elemental analysis results. The crystals were subjected to Fast Atom Bombardment (FAB) mass spectrometry. The [MH^+] ion was apparent at m/z: 239.

The direct coupling of indole (**85**) with the chloromethyl ether (**66**) in anhydrous DMF at room temperature was undertaken. Although most of the indole failed to react, a multitude of minor compounds were seen to form by TLC. These were produced in such small quantities and were so difficult to separate that their characterisation was not feasible. The reaction was repeated using anhydrous THF as solvent with initial cooling of the reaction to 5°C for two hours with similar results to those previously described. This was attributed to the indole ring's susceptibility to multiple alkylation mostly in the C-3 and C-2 positions [163] resulting in a mixture of products. Excellent yields have been reported for the preparation of similar molecules by coupling persilylated purine and pyrimidine rings to chloromethyl ethers such as **66** using tetra-n-butylammonium iodide (TBAI) as a condensation catalyst [164, 165]. This procedure was applied to

Scheme XVI



1) HMDS, $(NH_4)_2SO_4$

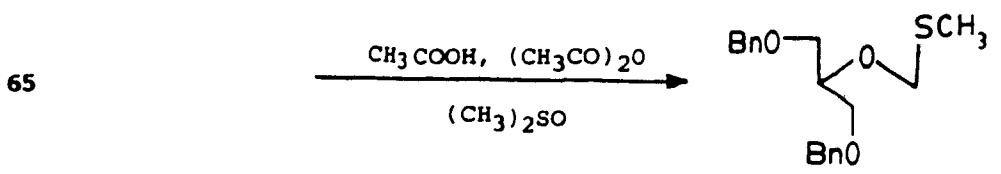
2) **66**/TBAI, THF

69

the indole coupling and also to that of benzimidazole for purposes of comparison. The heterocyclic base was suspended in hexamethyldisilazane (HMDS), ammonium sulfate was added as catalyst and the solution was refluxed under argon until the base was completely dissolved (Scheme XVI). Following the removal of excess HMDS, the persilylated base was dissolved in THF and treated with **66** in the presence of TBAI. Reactions were stirred for 15 hours at room temperature. The coupling of indole proved unsatisfactory as the solution became intensely coloured and a multitude of products were formed. Conversely, the protected benzimidazole analogue (**69**) was isolated in 60% yield and no unreacted benzimidazole was recovered. The lower yield of **69** obtained by the HMDS mediated procedure versus that obtained for direct coupling with **66** in DMF might be due to the loss of some persilylated benzimidazole during the vacuum distillation of excess HMDS. In fact, this procedure could not be used for the preparation of the imidazole analogue (**67**) as considerable loss of the low boiling persilylated imidazole occurred at this stage of the reaction.

In the preparation of trihydroxy acyclic purine and pyrimidine nucleoside analogues, Ogilvie and co-workers reported efficient coupling results using an alkylthiomethyl ether [166]. This intermediate is less reactive than the chloromethyl ether and has been useful in limiting the number of side products obtained in condensation reactions with isoguanine, a base susceptible to non selective, multiple alkylations [167]. The 2-methylthiomethyl-1,3-dibenzyloxypropyl ether (**84**) was used in an attempted synthesis of the indole analogue. Compound **84** was prepared in 47% yield by a procedure described by Pojer *et al.*[168] (Scheme XVII). A solution of 1,3-dibenzyloxy-2-propanol (**65**) in anhydrous dimethylsulfoxide with acetic acid and acetic anhydride was stirred at room temperature for

Scheme XVII

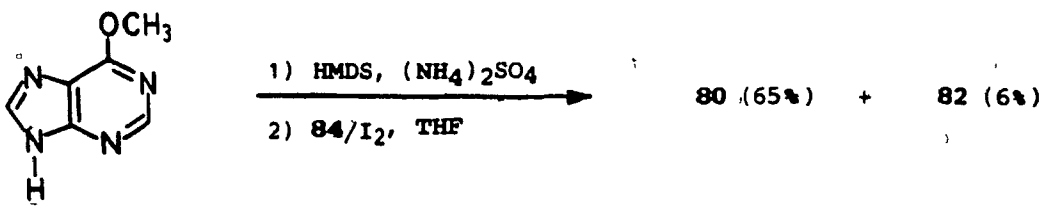


84

48 hours. The product which was purified by chromatography and fractional distillation in a Kugelrohr apparatus was used directly for coupling with the nitrogenous bases.

The condensation procedure was performed with both indole and 6-methoxypurine for purposes of comparison. The persilylated bases, prepared as previously described were dissolved in anhydrous THF and reacted with **84** (Scheme XVIII). The thiomethyl ether was activated by introduction of iodine to the reaction mixture. The subsequent addition of indole resulted, as before, in the production of a multitude of minor products which were not isolated or characterised. The reaction of 6-methoxypurine with the thiomethyl ether gave the desired 9-isomer (**80**) (65% yield) and the 7-isomer (**82**) (6% yield). The yield of **80** obtained is superior to that reported for the direct coupling of the non-silylated base with chloromethyl ether (**66**). More importantly, although the overall coupling yield for both isomers is inferior, the persilylation ensured a more favourable

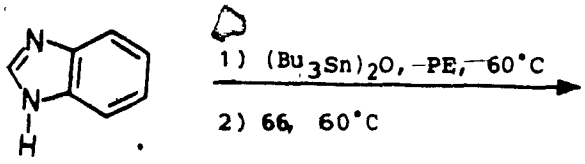
Scheme XVIII



N-9 to N-7 isomer ratio i.e. 10:1 versus 3:2 for the direct coupling route. It is possible that this is due to steric hindrance to silylation in the N-7 position by the methoxy group at C-6.

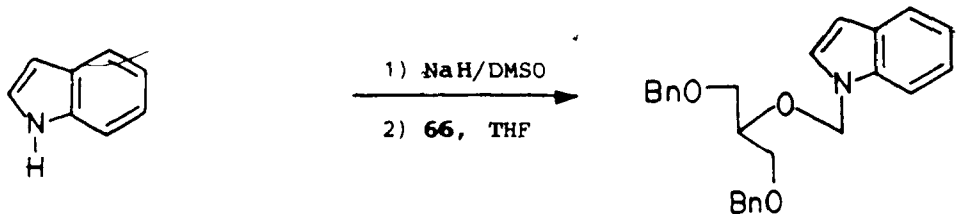
Another attempt at coupling the acyclic moiety described to indole was suggested by a procedure developed by Balasubramanian et al.[169] in which exclusive N-alkylation is obtained via an organotin intermediate. The reaction with indole and benzimidazole was attempted by this route. The nitrogenous base was first reacted with bis(tri-n-butyltin) oxide in petroleum ether. The water formed was removed from the refluxing solution using a Dean Stark apparatus. The organostannyl compound was not isolated and was treated directly with chloromethyl ether (66). The reaction with indole resulted once again in a multitude of intensely coloured minor products which were not identified. The benzimidazole analogue (69) was prepared in 67% yield by this procedure (Scheme XIX).

Scheme XIX



The preparation of several N-substituted indoles has been achieved by Sundberg and Russell by treatment of the sodium salt of indole with alkyl halides [170]. This approach was undertaken. The methylsulfenyl carbanion was first generated by treatment of anhydrous dimethylsulfoxide (DMSO) with sodium hydride at 75-85°C and subsequently reacted with indole (Scheme XX). The chloromethyl ether (66) was added and the reaction was allowed to proceed at room temperature for three hours. After work-up, excess DMSO was eliminated by vacuum distillation and the product was

Scheme XX



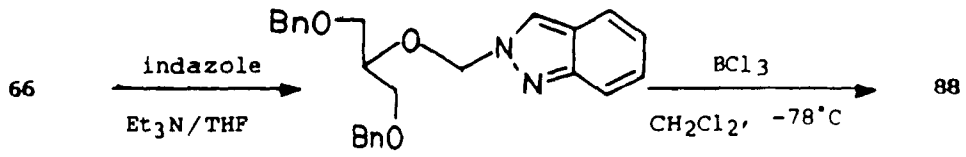
85 86

purified by column chromatography. The desired 1-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]indole (**86**) was isolated as an oil (82.8% yield). The product was stored at 0°C under argon atmosphere as the colourless oil darkens progressively to black when exposed to air and left at room temperature. Debenzylation with boron trichloride was attempted repeatedly but invariably resulted in decomposition during work-up of the reaction. Several purple coloured compounds with similar chromatographic properties were formed. ^{1}H NMR analysis of the mixture confirmed the absence of the CH_2N signal (5-6 ppm) characteristic of the heterocyclic analogues.

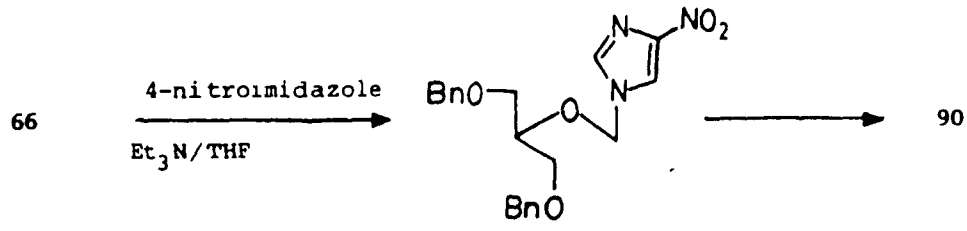
The attempted preparation of the N-1 analogue of indazole by direct coupling with **66** (Scheme XXI) resulted in the formation of 2-[[2-Benzyloxy-1-(benzyloxy-methyl)ethoxy]methyl]indazole (**87**) in 94% yield. Although the N-1H tautomer of indazole is predominant [171], none of the desired N-1 substituted indazole was recovered. The presence of three minor side products was detected by TLC but their isolation was not feasible due to their similar chromatographic properties and the small quantities produced. Upon repeating the procedure in anhydrous DMF, the recovered yield decreased to 60%. The purification was also rendered difficult because of the similar R_f values of the product and the hydrolysed chloride (**65**).

Although antiviral activity has not been reported for N-2 substituted pyrazolopyrimidine analogues, the compound was debenzylated with boron trichloride in anhydrous dichloromethane to yield 89% of the acyclic indazole derivative (**88**) as a yellow oil. Attempts to crystallise the oil from a variety of solvents proved unsuccessful. The UV absorption profile of **88** shows maxima at 275 and 293 nm. These values are within the ranges of those for 2-methylindazole (275, 295 nm) and differ from those reported at pH 7 for 1-methylindazole (254, 292 nm) [172]. NMR data is discussed in section 1.2.2. Results from elemental analysis were within acceptable limits if half a mole of water is added. The presence of water, introduced during attempts at crystallisation was confirmed by ^{1}H NMR and the integration was consistent with analysis results.

Scheme XXI



87

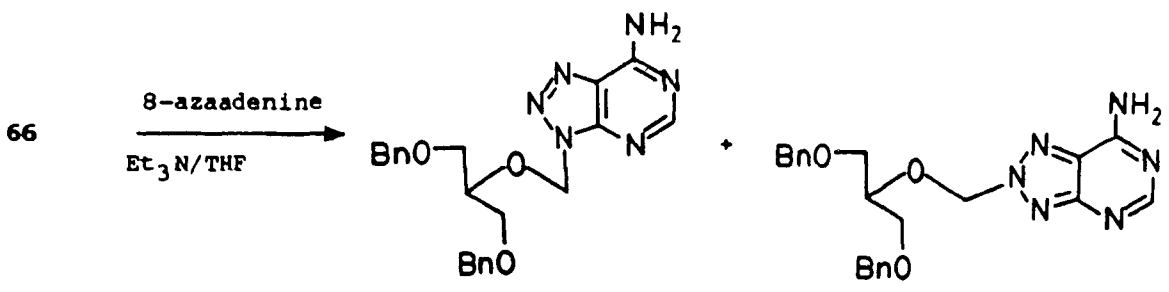


89

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-4-nitroimidazole (**89**) was prepared in the same manner as **87** in excellent yield (95%) despite the limited solubility of 4-nitroimidazole in THF. The yellow oil obtained was treated with boron trichloride affording 74% of the desired analogue (**90**) as a colourless oil. This was easily crystallised from methanol/water to white needles. As in the case of the imidazole analogue (**68**), elemental analysis results were within acceptable limits when calculated with the addition of half a mole of water. These results are consistent with the integration of the water peak observed by ^1H NMR. The structure of **90** was further confirmed by NMR (section 1.2.1).

The coupling of 8-azaadenine with the chloromethyl ether (**66**) was attempted by direct reaction in THF with triethylamine but resulted in multiple minor alkylations and almost complete recovery of the starting material. When the base was first persilylated then treated with **66** with TBAI in THF by the procedures previously described, both the desired 9-isomer and the 8-isomer were obtained in yields of 45% and 11% respectively. These were distinguished by their characteristic UV absorptions consistent with those reported for N-9 and N-8 substituted 8-azaadenines 154, 173, 174. The 9-isomer shows a maximum at 280 nm; the 8-isomer at 296

Scheme XXII



91 (45%) **92** (11%)

nm. The two products were further differentiated by their ^{13}C NMR spectra discussed in section 1.2.3. Debenzylation of the 9-isomer using boron trichloride was not successful despite repeated attempts. Cleavage of the pseudo-glycosidic bond (C-1',N) was found to occur.

1.2 NMR Characterisation of the Heterocyclic-Ring Analogues

The heterocyclic-ring analogues of BIOLF-62 were characterised by both ^{1}H and ^{13}C NMR. Proton signals were assigned by analogy with the values reported in the literature and by selective decouplings. Carbon signals were also assigned according to literature values for analogous compounds and by using the DEPT (Distortionless Enhancement by Polarisation Transfer) and the ^{1}H-^{13}C HETCOR (hetero-correlation) techniques described in the appendix (sections I and III).

1.2.1 The Imidazole and 4-Nitroimidazole Analogues

^{1}H Chemical shifts of the imidazole and the 4-nitroimidazole analogues are reported in Table III. A first observation of the ^{1}H NMR spectra of the benzyl protected analogues **67** and **89** reveals the disappearance of the base NH signal (δ = 10-13 ppm). The H-2 signal is shielded in the protected 4-nitroimidazole analogue (**89**) relative to the parent imidazole compound (0.36 ppm) and the H-5 signal is also shifted upfield (0.46 ppm). These effects are possibly due to steric interactions of H-2 and H-5 with the benzyl groups. As the data for compound **90** show, the shielding effects disappear upon removal of the benzyl groups.

TABLE III

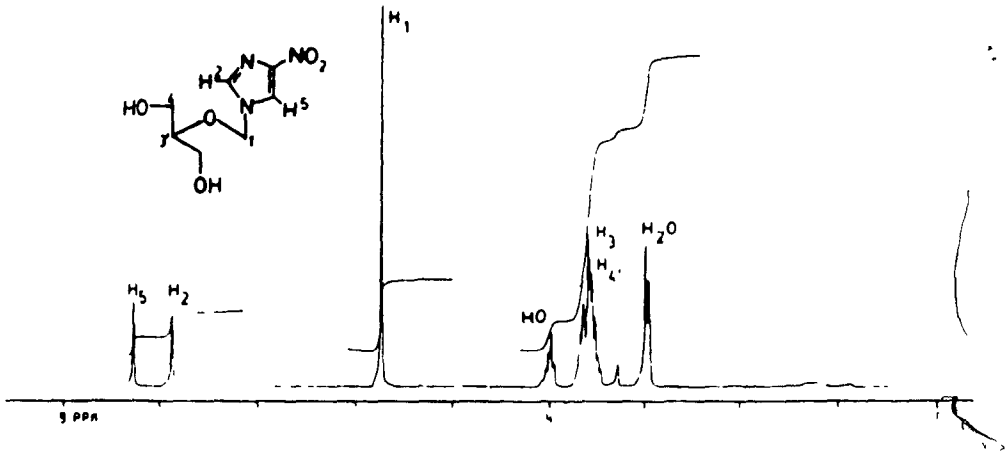
^{1}H Chemical Shifts of the Imidazole and 4-Nitroimidazole Analogues

H	67[a]	68[b]	89[a]	90[b]
2	7.55 (bs)	7.72 (bs)	7.49 (bs)	7.89 (bs)
5	7.04 (bs)	7.24 (bs)	7.84 (bs)	8.30 (bs)
4	6.98 (bs)	6.94 (bs)	--	--
1'	5.42 (s)	5.56 (s)	5.46 (s)	5.72 (s)
CH2Ph	4.41 (s)	--	4.42 (s)	--
OH	--	4.00 (m)	--	4.00 (m)
3'	3.73 (m)	3.52 (m)	3.81 (m)	4.00 (m)
4'	3.45 (m)	3.52 (m)	3.50 (m)	3.60 (m)

Spectra recorded on a Varian XL-200 instrument in a) $CDCl_3$ (δ = 7.24); b) acetone-d6 (δ = 2.17). δ reported in ppm relative to TMS.

Figure 5

^{1}H NMR of the 4-Nitroimidazole Acyclic Analogue in acetone-d6



The ^{1}H NMR shifts of the acyclic moiety protons are analogous to those of the ribose ring. The farthest downfield signal observed is a singlet which corresponds to the methylene protons of the pseudo-glycosidic bond (H-1'). The signals of the CHO (H-3') and CH_2CH (H-4') protons which correspond respectively to the H-4' and H-5' of ribose, are observed in the characteristic regions for these signals. In the deprotected imidazole analogues (68, 90), the chemical shift difference between the two

signals is not significant (0-0.6 ppm). These protons give rise to second order splitting patterns due to their involvement in an ABX system. The two H-4' protons of each methylene group are diastereotopic and hence exposed to slightly different magnetic environments (Figure 5). The H-4' splitting pattern is sometimes further complicated by coupling of H-4' to the hydroxyl protons which disappears with deuterium exchange.

TABLE IV

^{13}C Chemical Shifts of the Imidazole and 4-Nitroimidazole Analogues

C	67[a]	68[b]	89[a]	90[b]
2	139.69	139.29	138.09	138.34
4	126.36	122.12	152.16	147.53
5	129.76	131.02	126.75	121.34
3'	78.40	81.04	84.36	82.29
1'	75.60	77.01	83.46	78.29
CH2Ph	74.73	---	77.56	---
4'	72.04	62.78	73.97	62.70

a) Spectra recorded in CDCl3 (δ = 77.0) on a Varian XL-200 instrument.
b) recorded in CD3OD (δ = 49.0) on a Brüker WH-90 instrument.
δ reported in ppm relative to TMS.

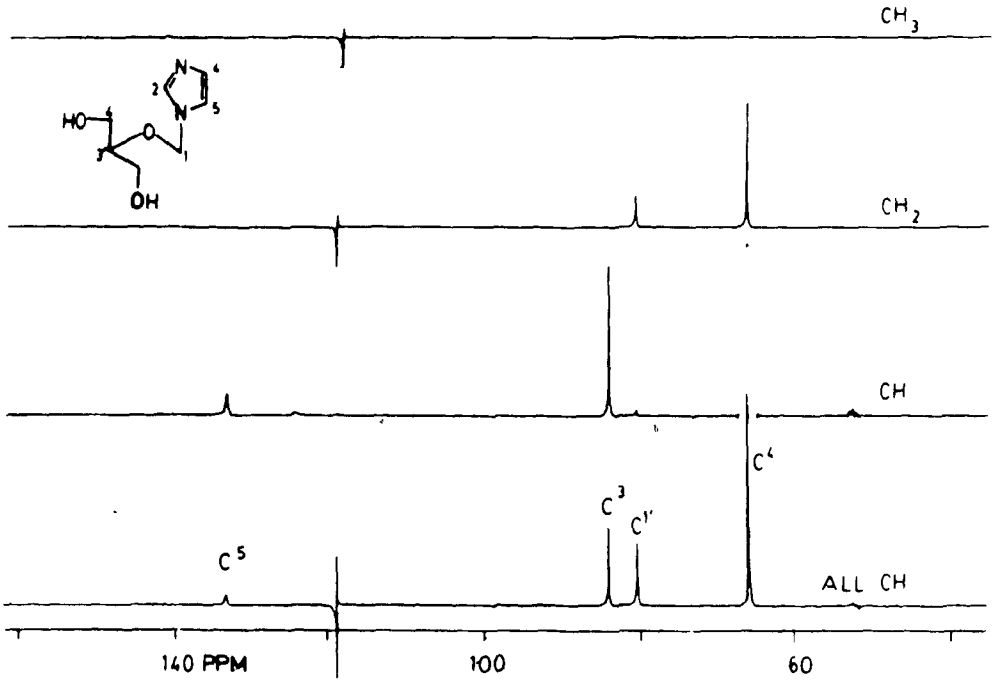
The assignment of ^{13}C chemical shifts of the imidazole analogues, reported in Table IV, was rendered difficult as no signal appeared for C-2 and C-4. However, C-2 and C-4 signals appeared as sharp peaks in the ^{13}C spectra of imidazole, 4-nitroimidazole and 1-methylimidazole. When pulse angles were increased and delay times shortened, two broadened signals were observed for the fast relaxing C-2 and C-4 of each analogue.

The base carbon signals of the protected analogues (**67**, **89**) are all deshielded relative to the peaks of the parent imidazole compounds. The effect of N-1 substitution is most marked at C-5 which moves downfield by 7-8 ppm. The C-2 and C-4 signals are shifted downfield by approximately 2-4 and 4-5 ppm respectively. The C-2 and C-5 peak positions are consistent

with those reported for 1-alkyl imidazole derivatives [175, 176]. Spectra of the fully deprotected analogues (68, 90) were recorded in deuterated methanol because of their limited solubility in other solvents.

Figure 6

^{13}C ~~NMR ADEPT~~ Spectra of the Imidazole Acyclic Analogue in Methanol-d4



The carbon peaks of the acyclic portion of the deprotected analogues (68, 90) were assigned using ^{13}C DEPT NMR spectroscopy [177] (Figure 6). This pulse sequence (described in the Appendix, section I), not only enhances the sensitivity of the carbon signals but is amenable to ADFPT editing which generates subspectra sorted according to carbon multiplicities. As these analogues contain no CH_3 groups, no signals are apparent in the first spectrum. Of the two signals visible in spectrum 2, the farthest upfield was identified as C-4' as the chemical shift of this carbon is the most affected by removal of the benzyl groups and appears in

the same region as the equivalent C-5' of ribose. The other CH_2 signal is farthest downfield as is the case for the ribose C-1' (δ = 90 ppm) and is readily assigned. The CH groups are shown in the third spectrum thus allowing unequivocal C-3' identification. The chemical shift of this carbon is consistent with that of the corresponding ribose C-4'. The signals of all proton bearing carbons appear in the fourth spectrum.

1.2.2 The Bicyclic Analogues

The ^{1}H and ^{13}C chemical shifts of the bicyclic analogues are summarised in Tables V and VI.

The benzimidazole base signals of compound **70** were assigned by selective decouplings. The complex splitting patterns of H-4 and H-7 reveal coupling to their respective vicinal protons, H-5 and H-6 and longer range coupling between H-4 and H-6 as well as between H-7 and H-5 (see Figure 7 for ring numbering). A slight shielding of the H-6 and H-7 signals is observed in both the protected (**69**) and debenzylated (**70**) analogues relative to benzimidazole. Similarly in analogues **71** and **72**, the H-2 and H-6 signals are slightly shielded relative to 7-azaindole. In both compounds, the small splitting (1.1 Hz) between H-6 and H-4 is resolved.

The carbon signals of analogues **70** and **72** were also assigned using ^{13}C DEPT NMR. The ambiguity existing between the C-5 and C-6 signals of compound **70** was not resolved with this technique as both carbons bear one proton and are not differentiated. A similar problem occurs when attempting to assign C-8 and C-9 of compound **72**, as neither carbon bears a proton and thus they do not appear in the DEPT spectrum. The effect of N-1 substitution on the bridgehead carbons of these two compounds is similar to that reported for the alkylation of benzofuranes [178] and purines in the

TABLE V

^{1}H Chemical Shifts and Coupling Constants (J) of the Bicyclic Analogues

H	Benzimidazole (70)[a]	7-Azaindole (72)[a]	Isatin (74)[b]	Indazole (88)[b]
2	7.88 (s)	7.29 (d) (3.4)	--	--
3	--	6.49 (d) (3.4)	--	8.43 (s)
4	7.70 (m)	7.93 (dd) (7.9, 1.1)	7.59 (dd) (7.6, 0.9)	7.60 (dd) (8.8, 1.6)
5	7.28 (m)	7.11 (dd) (7.6, 4.8)	7.20 (m) (7.7, 1.2)	7.25 (m) (8.1, 1.2)
6	7.28 (m)	8.29 (dd) (4.7, 1.1)	7.70 (m) (7.7, 1.2)	7.02 (m) (7.2, 1.0)
7	7.50 (m)	--	7.30 (d) (7.6. 1.0)	7.68 (dd) (8.1, 0.8)
1'	5.69 (s)	5.84 (s)	5.35 (s)	5.93 (s)
3'	3.62 (m)	3.83 (m)	3.68 (m)	3.79 (m)
4'	3.62 (m)	3.66 (m)	3.68 (m)	3.55 (m)

Spectra recorded on a Varian XL-200 instrument in a) CDCl3; b) acetone-d6. δ reported in ppm relative to TMS. Coupling constants (J) reported in Hz.

N-1, N- 3, N-7 and N-9 positions [176] i.e. the α carbon, in this case C-8, has moved upfield (ca 3.5 ppm) while the β carbon, C-9, is deshielded (ca 6 ppm).

The ^{13}C chemical shift positions of the acyclic portion of analogues **70** and **72** show only one marked difference from those of the imidazole analogues i.e. a slight upfield shift of the C-1' signal by approximately 3 ppm. The chemical shift of C-1' is similar to that observed for the methyl signal of C-7 methylated benzofuranes (peri effect) [178] and for the methylene signal of N-3 or N-9 substituted purines [176]. The shielding is

TABLE VI

^{13}C Chemical Shifts of the Bicyclic Analogues

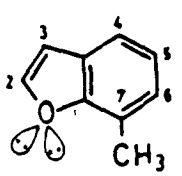
C	Benzimidazole (70)	7-Azaindole (72)	Isatin (74)	Indazole (88)
2	145.02	148.69	160.40	---
3	---	101.98	184.39	141.62
4	120.10	122.91	128.23	128.19
5	124.69 (a)	130.03 (b)	125.76	123.18 (c)
6	123.99 (a)	130.62 (b)	139.47	121.99 (c)
7	112.02	---	113.09	117.94
8	144.11	143.46	151.89	150.31
9	134.67	117.46	119.13	125.87
3'	80.88	80.56	81.83	82.02
1'	75.27	74.52	70.90	82.39
4'	62.76	62.59	62.58	62.54

Spectra recorded in CD_3OD on a Varian XL-200 instrument.
a) b) c) Signals might be inverted. δ reported in ppm relative to TMS.

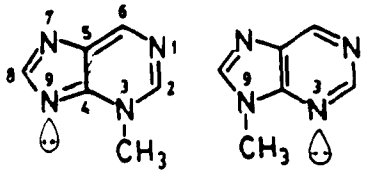
attributed in both cases to steric crowding by the lone pair (or the hydrogen atom) on the γ nitrogen or oxygen atom (Figure 7). Although steric crowding by a hydrogen atom on C γ also causes upfield shifts, these are slightly smaller (ca 2 ppm) than the shielding by lone pair electrons and this difference can be useful in distinguishing positional isomers [176, 178]. However, the C-1' shift of the benzimidazole analogue is only 0.75 ppm downfield of the C-1' signal of the 7-azaindole analogue. This difference is smaller than might be predicted on the basis of previous results as in the former case, C-1' is crowded by a hydrogen atom (H-7) and in the latter, by the lone pair of N-7 (Figure 7). However, the C-1' signal of the benzimidazole analogue might appear farther upfield as a result of the continued presence of a shielding γ gauche effect which has moved from N-7 to N-3. In the isatin analogue (74) the shielding of C-1' relative to its position in the imidazole analogues is even more pronounced (6 ppm) due to the additional crowding by the C-2 substituent (Figure 7).

Figure 7

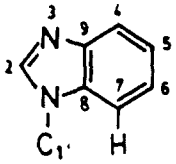
Shielding Effects on C-1' in Substituted Heterocycles



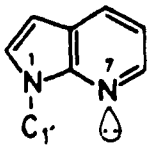
Benzofuran



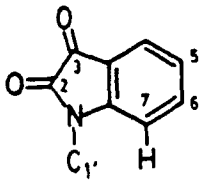
Purine



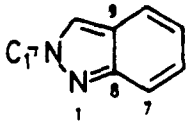
70



72



74

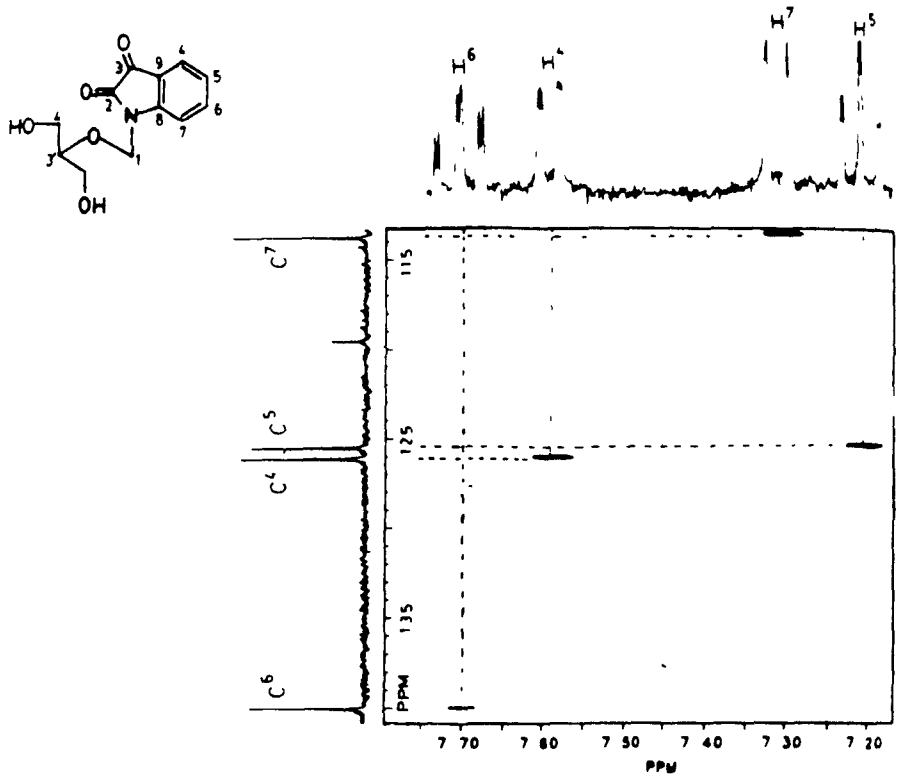


88

The assignments of both the ^{1}H and ^{13}C peak positions for the base portion of the isatin analogue (74) were slightly ambiguous. Although the carbons bearing hydrogen atoms were identified by ^{13}C DEPT NMR and the ^{1}H spectrum was elucidated by selective decouplings, the differentiation of the C-4 and C-5 signals as well as that of the H-6 and H-5 signals required the use of ^{1}H-^{13}C Heterocorrelation. The resulting two dimensional spectrum is reproduced in Figure 8. The relevant portion of the proton spectrum appears along the horizontal axis while the carbon spectrum is reproduced along the vertical axis. The C-6 signal which should not be shifted significantly was assigned by analogy to the reported parent isatin shifts [175] thus allowing the identification of the correlated H-6 signal. The other proton assignments were determined by ^{1}H decoupling experiments. The distinction of the C-4 and C-5 signals was established according to their correlations with the appropriate ^{1}H signals.

Figure 8

^{1}H-^{13}C Hetero-Correlated Spectrum of the Isatin Acyclic Analogue in Methanol-d4



The ∝ bridgehead carbon, C-8, is shifted upfield (1.2 ppm) and the bridgehead carbon, C-9 is shifted downfield (1.5 ppm) relative to the parent isatin signals. Although these effects are less important than those observed for the benzimidazole and 7-azaindole analoques, they conform to the trend reported for benzofurane and purine substitution.

In addition to the previously mentioned UV data concerning N-2 substitution in the indazole analogue (88), there is supporting evidence in both the ^{13}C and ^{1}H spectra. The most notable effect is the downfield shift of more than 12 ppm of the C-1' signal relative to its position in the N-1 substituted analogues. This C-1' signal appears even farther downfield than in the imidazole analogues as the shielding effect of the

hydrogen atom or lone pair in the 7-position of the base is no longer involved (Figure 7). In addition to this, a deshielding β effect due to N-1 may affect the C-1' shift. ^{13}C DEPT NMR confirms that in compound **88**, the C-1' signal is at lower field than the C-3'. The bridgehead carbon signals (C-8 and C-9) are both deshielded relative to the analogous signals of nonsubstituted indazole. Both these carbons are α to the substituted nitrogen and are shifted to lower field as were the α carbons (C-9) of the benzimidazole and 7-azaindole analogues. Had substitution occurred at N-1, a shielding of C-8 would have been expected in analogy with the signal observed in compounds **70**, **72** and **74**. Also, the slight shielding of H-6 and H-7 observed in the ^{1}H spectrum of compounds **70** and **74**, relative to the unsubstituted bases, does not occur in this analogue which confirms that substitution has not occurred at the N-1 position.

1.2.3 The Purine Analogues

As discussed in section 1.1, 6-methoxypurine reacted in both the N-9 and N-7 positions to yield the protected analogues **80** and **82**. The 8-azapurine base reacted in both the N-9 and N-8 positions to yield **91** and **92**. In addition to the UV data previously presented, the ^{1}H and ^{13}C chemical shifts reported in Tables VII and VIII help distinguish between the positional isomers.

The N-1 substituted heterocycles discussed in section 1.2.2, show a shielding of the H-7 and H-6 signals relative to those of the free base. In the protected 9-isomer purine analogues (**80**, **91**), the analogous proton, H-2, is also shielded though to a lesser extent. This proton is farther downfield in both the 7-isomer of 6-methoxypurine (**82**) and the 8-isomer of 8-azaadenine (**92**) due to the lesser crowding. These results are consistent

TABLE VII

^{1}H Chemical Shifts of the 6-Methoxypurine and 8-Azaadenine Analogues

H	80[a]	82[a]	81[b]	91[a]	92[a]
8	8.53 (s)	8.61 (s)	8.52 (s)	--	--
2	8.04 (s)	8.14 (s)	8.47 (s)	8.41 (s)	8.45 (s)
R-6	4.17 (s)	4.05 (s)	4.09 (s)	7.23 (m)	7.24 (m)
1'	5.79 (s)	5.83 (s)	5.71 (s)	6.10 (s)	6.06 (s)
OH	--	--	4.58 (m)	--	--
CH2Ph	4.43 (s)	4.41 (s)	--	4.39 (s)	4.42 (s)
3'	4.02 (m)	3.88 (m)	3.57 (m)	3.88 (m)	3.86 (m)
4'	3.49 (m)	3.49 (m)	3.30 (m)	3.50 (m)	3.49 (m)

All spectra recorded on a Varian XL-200 instrument in a) CDCl3; b) dmso-d6 (δ = 2.39). δ reported in ppm relative to TMS.

with those reported for the N-9 and N-7 isomers of methylpurine [179]. The methoxy protons (R-6) of **82** are more shielded in the 7-isomer reflecting the increased crowding. No difference is noted in the amine protons (R-6) of the 8-azaadenines. Although the ^{1}H shift differences presented above are informative, they are generally small and do not provide conclusive evidence for isomer identification as does ^{13}C NMR data.

The 6-methoxypurine analogues are easily distinguished by ^{13}C NMR. The characteristic shifts of the bridgehead carbons (C-4, C-5) in N-7 and N-9 substituted isomers relative to those of the free purine bases have been reported in detail [176, 179, 180]. An upfield shift (3.4 ppm) of C-4 occurs upon alkylation of N-9 whereas this signal moves downfield (5.1 ppm) in the N-7 isomer. Conversely, the C-5 signal shifts downfield (3 ppm) for 9-alkylpurine and upfield (4.7 ppm) for the 7-isomer. These observations were applied to the distinction of the protected 6-methoxypurine analogues (see Figure 9 for ring numbering). In the compound identified by UV as the 9-isomer (**80**), the C-4 signal is shifted upfield whereas it moves to lower field in the 7-isomer (**82**) which was also characterised by UV. These observations are consistent with the reported effects on the

TABLE VIII

^{13}C Chemical Shifts of the 6-Methoxypurine and 8-Azaadenine Analogues

C	80[a]	82[a]	81[a]	91[b]	92[b]
6	162.13	162.23	160.33	157.98	162.69
2	153.49	153.23	152.18	157.32	155.61
4	153.22	158.89	151.86	150.51	153.03
8	144.92	148.21	144.04	---	---
5	121.83	113.79	120.52	139.27	138.92
3'	79.32	78.89	80.87	78.43	77.94
1'	74.19	77.54	72.25	75.40	79.94
CH2Ph	74.19	74.25	---	73.43	73.59
4'	71.33	71.39	60.97	70.83	70.48
OCH3	54.83	54.93	53.94	---	---

Spectra recorded in a) CD3OD; b) acetone-d6 (δ = 29.8) on a Varian XL-200 except **80** and **82** recorded on a brüker WH-90 instrument. δ reported in ppm relative to TMS.

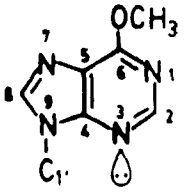
C-4 chemical shifts of purines following N-7 or N-9 substitution. In the 7-isomer, C-5 is α to the substituted nitrogen and its signal appears upfield (8.04 ppm) of its position for the 9-isomer. This difference is also consistent with the C-5 shifts reported for N-7 and N-9 substituted purines.

In these molecules, the C-4 signal is difficult to distinguish from that of C-2 using ^{13}C DEPT NMR. Although C-4 bears no protons and does not appear in the DEPT spectrum, the chemical shifts of C-2 and C-4 are almost identical and cannot be differentiated. However, in decoupled ^{13}C NMR, bridgehead carbon signals are known to be of lesser intensity due to the saturation effect resulting from their long relaxation times [181]. Thus, these can be distinguished from proton bearing carbons. This effect has also been observed for the C-4 and C-5 signals of purines and the C-6 signal when this position is substituted (R-6 $\neq$ H) [182]. From this information, the signal of lesser intensity was assigned to the bridgehead carbon, C-4.

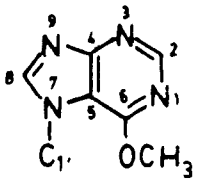
The ^{13}C chemical shifts of C-1' are also characteristic for each isomer and are consistent with the reported CH_3 shifts in the 7-methyl and 9-methyl-6-methoxypurines [182]. The greater shielding of C-1' in the 9-isomer (3.4 ppm) may be partially explained as follows. In the 9-isomer (80), C-1' is crowded by the lone pair on N-3 and might also be shielded by γ gauche effects from N-7 and N-3 (Figure 9). The C-1' in the 7-isomer (82) is subjected to a lesser crowding from O-6 and only one γ gauche interaction resulting from a nitrogen atom (N-9) is possible.

Figure 9

Contributing Shielding Effects in the 6-Methoxypurine Analogues



80



82

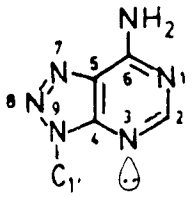
The 8-azaadenine isomers may also be differentiated by ^{13}C NMR, in addition to the UV data previously presented. As expected, the C-4 signal is upfield (2.52 ppm) in the N-9 isomer relative to its position in the N-8 isomer. The reverse effect is observed for the C-5 peak positions (0.35 ppm). The C-4 chemical shift differences are also consistent with those reported for the 8-azaadenine base signals following N-8 and N-9 substitution with a ribose ring [183, 184].

The C-1' chemical shifts also confirm the identification of the isomers (Figure 10). The C-1' signal is upfield in the N-9 isomer (2.54 ppm) compared to the N-8 compound. This is due in part to lone pair

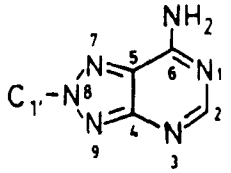
effects from N-3 as well as to possible γ gauche effects from N-7 and N-3 leading to shielding of C-1'. Furthermore, in the N-9 isomer, C-1' is exposed to a single β deshielding effect (N-8) while it is exposed to two in **92** (N-7, N-9).

Figure 10

Contributing Shielding Effects in the 8-Azaadenine Analogues



91



92

1.3 Biological Testing Results

The deprotected acyclic analogues were sent for biological testing. Compounds **68**, **70** and **72** were tested in vitro against strains of HSV-I, HSV-II and cytomegalovirus grown in human fetal lung (HFL) cell monolayers. The compounds were found inactive up to concentrations of 100 μg/mL. The hydroxymethylisatin (**78**) produced in the first debenzylation with boron trichloride was also tested. This compound exhibited marked toxicity to the host cells.

Analogues **74**, **88** and **90** were tested against strains of HSV-I and HSV-II grown in Vero cell monolayers. No antiviral activity was observed up to concentrations of 100 μg/mL.

Analogue **81** has been tested previously against both HSV strains, cytomegalovirus, feline and canine herpesviruses, bovine herpes virus-4 and equine herpes virus-1 but was found inactive up to concentrations of 100μg/mL [157].

1.4 Conclusion

The direct coupling procedure used to link the nitrogen heterocycles to the acyclic chloromethyl ether (**66**) has proved efficient for bases possessing one reactive resonance structure such as 7-azaindole and isatin or existing in two equivalent tautomers such as imidazole and benzimidazole. The coupling of 4-nitroimidazole also resulted in a single product possibly due to the electron withdrawing effect of the nitro group on N-3 and to the greater crowding at this position.

The procedure is less efficient for the linking of a base such as 6-methoxypurine as its imidazole ring exists in two tautomeric forms (7-H, 9-H). The population of the 9-H tautomer of 6-methoxypurine was estimated at 68% by ^{13}C chemical shift substituent parameter studies by Grant, Townsend *et al.*[182]. However, the relative percentage of the protected 9-isomer analogue (**80**) obtained by direct coupling of the base to **66** is slightly lower (60%). The compound is obtained in greater proportion by first persilylating the base with HMDS. The trimethylsilylated 9-isomer is preferred, possibly due to steric hindrance of the N-7 position by the 6-methoxy group.

The 8-azaadenine coupling presented a similar problem. Although the free base is known to exist in a single tautomeric form (9-H) [183], multi-

ple alkylations occurred in the reaction of 8-azaadenine with the acyclic chloromethyl ether (66). Persilylation the base favoured the reaction at the N-9 position over the N-8 and eliminated reaction with the exocyclic amine group. However, the isomer ratio was less advantageous than for 6-methoxypurine (4:1 versus 10:1) possibly reflecting the absence of steric hindrance to silylation in the N-8 position.

The direct coupling procedure was not useful for obtaining the desired N-1 isomer of the indole analogue. Preliminary persilylation did not significantly influence the number of multiple alkylations of the base. Generation of the N-1 anion with the methylsulfenyl carbanion was necessary to obtain reaction with the chloromethyl ether at the desired site.

The N-1 isomer was not isolated by direct coupling of indazole with the acyclic chloromethyl ether (66). Although the 1H tautomer is predominant, the sole product isolated was the N-2 substituted compound. In analogy with the results previously described for the 6-methoxypurine and 8-azaadenine couplings, persilylation of the base might increase the reaction at the N-1 position. This route has not been pursued further at this time.

The nuclear magnetic resonance properties particularly those of ^{13}C have been useful in differentiating positional isomers of the acyclic analogues. The qualitative interpretation of chemical shift displacements of the heterocyclic carbons and the C-1' in conjunction with other spectroscopic data is a reliable method of characterising the compounds. The carbon chemical shifts of the heterocyclic bases are dependant on multiple factors notably charge polarisation effects and C-C bond lengths and cannot be easily explained [176, 184]. Nevertheless, although the substituent effects are not additive and cannot be predicted, the trends in

specific carbon shifts, notably the bridgehead carbons, are consistent from one analogue to the other and in accord with the chemical shift variations reported for similar compounds.

The absence of biological activity for all analoques prepared underlines the importance of the nature of the base residue to the activity of such acyclic antiviral agents as acyclovir and BIOLF-62. Despite the greater activities reported for these acyclic purine systems over those of arabinose, ribose or deoxyribose nucleosides, the replacement of the naturally occurring purine bases has resulted in complete loss of antiviral activity against the herpes simplex viruses. This is particularly noteworthy in the case of the 6-methoxypurine analogue (81) which, among the acyclic compounds prepared, is the most similar to BIOLF-62. It is also interesting that the isatin analogue, although inactive, is non-toxic to the host cell whereas the replacement of the acyclic portion of 74 by a hydroxymethyl group (78) results in severe toxicity to host cells. These effects further illustrate the delicate balance which must be achieved in the design of an effective antiviral agent.

CHAPTER TWO

Preparation of Protected Arabinoadenosine Nucleosides

2.0 Introduction

1-β-D-Arabinosyl thymidine and uracil nucleosides were first isolated in the early 1950's by Bergmann and Feeney from the Caribbean sponge, Cryptotethia crypta [185]. Arabinocytidine (araC) (18) [186] and arabinoadenosine (araA) (24) [137] were later synthesised as potential antitumour agents. Despite its cytotoxicity, araC was found effective in the treatment of certain leukemias while the activity of araA was most pronounced in the control of viral infections notably those of herpes simplex, vaccinia virus and cytomegalovirus [188-190]. During the same period, researchers in the United States (Parke, Davis & Company, The Southern Research Institute) observed the antiviral properties of an antibiotic fermentation concentrate of Streptomyces antibioticus and later identified the active agent as araA. The naturally occurring ribonucleoside epimer was then produced in large scale by fermentation processes and tested extensively in vitro and in vivo as an antiviral drug [51, 191, 192]. AraA was the first drug licensed in the United States for the treatment of systemic viral disease and the only drug approved for systemic treatment of HSV encephalitis [22]. AraA is active against a wide range of pathogens, mostly DNA viruses, some of which are listed in Table IX. The activity against the Rous sarcoma strain, an RNA tumour virus, is surprising as araA is generally ineffective against RNA viruses.

TABLE IX

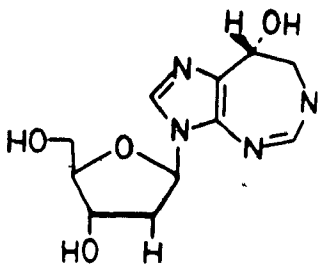
Some Viruses Inhibited by the Action of AraA

Herpes simplex I, II	poxviruses Vaccinia
Herpes simiae	Monkeypox
Varicella-zoster	Myxomavirus
Cytomegalo	Pseudorabies
Bovine rhinotracheitis	Fish lymphocystitis
Feline herpes	Polio II
Bovine herpes	Catfish herpes
Rabies	Rous sarcoma

Although the exact mode of araA antiviral action has not been completely elucidated and several mechanisms might be involved, it is known that the 5'-triphosphate derivative (araATP) is a competitive inhibitor of both viral and cellular DNA polymerases with a certain selectivity exhibited for the viral enzyme [193, 194]. The competitive action observed between araATP and deoxyadenosine triphosphate [195] is explained by the similarity of the α face of the araA sugar ring to that of the equivalent deoxynucleoside despite the presence of the 2'-hydroxyl group. Unfortunately, araATP is incorporated into the small quantities of DNA produced in both infected and healthy cells although to a lesser extent in the latter, hence the cytoxicity exhibited at high concentrations [196]. AraATP was originally thought to act as a chain terminator [197] but more recent studies have determined the nucleoside is uniformely distributed in the viral genome (complete set of genes) though it may serve to retard chain elongation [26]. In vitro resistance to araA has been partially linked to cell culture deficiencies of the activating kinase enzymes responsible for the monophosphorylation of araA [198]. The 5'-monophosphate derivative of araA (araAMP) which bypasses the initial phosphorylation step possesses the same activity as the parent compound [199]. In addition, araAMP is resistant to adenine deaminase [200]. In both animal and bacterial systems,

this enzyme rapidly converts araA to its hypoxanthine derivative (araH) (**25**) [201-203]. The potency of both araA and araH have been evaluated and while araA is the more active *in vitro* and the more cytotoxic [204], the *in vivo* efficacies of the two drugs are comparable [205]. The decreased activity of araA *in vivo* is due to its rapid deamination to the less potent araH by cellular and viral adenosine deaminase [206-209]. Although the effective half life of araA is prolonged by the antiviral activity of araH, large doses of the former are still required for the treatment of infection. However, the relative insolubility of araA hampers its systemic administration in large quantities. This problem has been partially solved by combination therapy. The *in vivo* activity of araA is enhanced when it is given along with adenosine deaminase inhibitors such as 2'-deoxycoformycin (**93**) [210] and erythro-9-(2-hydroxy-3-nonyl)adenine (**94**) [211]. However, these drug combinations lead to increased cytotoxicity [200, 212]. A modification of the sugar ring of araA, i.e. the replacement of the cyclic oxygen by a methylene group to yield the carbocyclic analogue (**95**), results in resistance to enzymatic deamination with retention of antiviral activity as well as stability to hydrolases and phosphorylases due to the absence of the labile glycosidic bond [213, 214]. Activity studies of 2'-substituted arabinoadenosines (**96**) reveal continued *in vitro* activity but decreased *in vivo* action relative to the parent compound despite the increased solubility and resistance to enzymatic deamination of the analogues [215, 216]. Similar results have been reported for 5'-substituted arabinoadenosines [217, 218]. The adenine deaminase resistance observed upon substitution of the 5' position of arabinonucleosides is consistent with the results obtained for similar modifications of ribonucleosides [164]. The effect of modifications of the base portion on antiviral activity have also been investigated.

Although replacement with 8-azaadenine (97) does not decrease *in vitro* activity, *in vivo*, the compound was found ineffective and severely toxic [155]. Substitution of the C-6 position with a hydroxylamine function (arabinosyl-N^6-hydroxyadenine, **98**) [220] results in resistance to adenosine deaminase, diminished toxicity and increased antiviral action.



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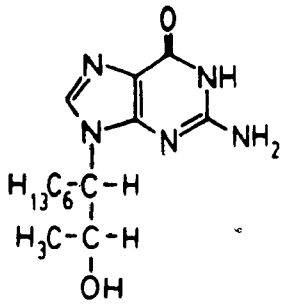

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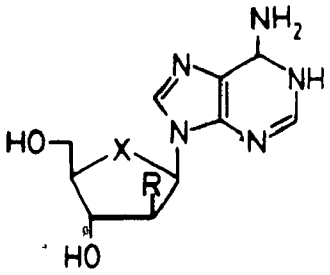
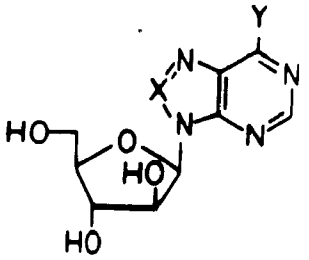


	X	R
95	CH_2	OH
96	O	F, Cl, OMe, OCH_2Ph, SCH_3, ONO_2



	X	Y
97	N	NH_2
98	CH	NHOH

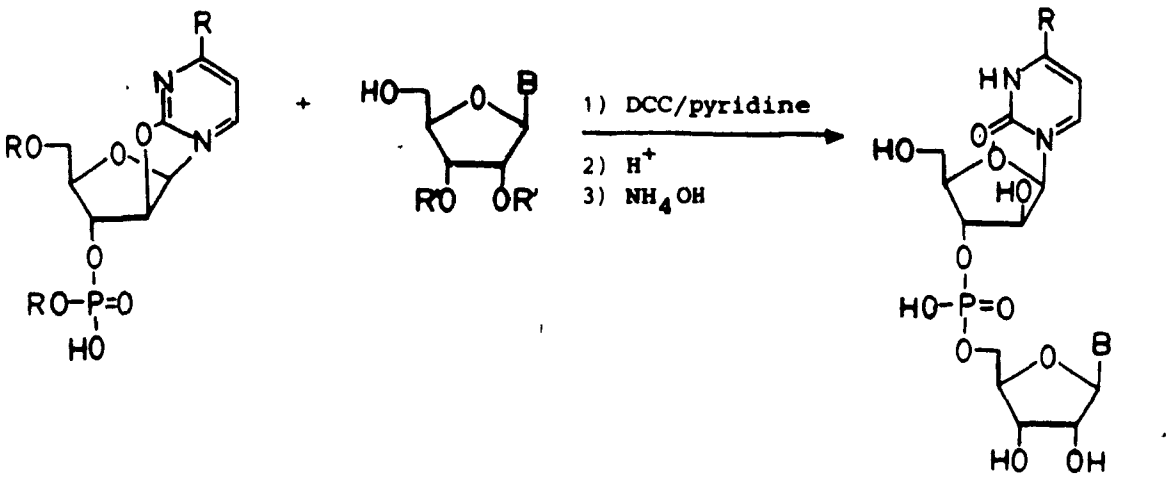
A different approach that might be envisaged to improve the biological action of araA is its incorporation into an oligonucleotide chain. The solubility, deamination and toxicity problems associated with the free nucleoside might be bypassed by this method. In addition to possible resistance to enzymatic deamination of the base and cleavage of the phosphodiester linkage, arabinonucleotides are expected to possess an inherent

stability comparable to that of deoxynucleotides. The arabinonucleotide phosphodiester, unlike the ribonucleotide linkage, cannot be cleaved by formation of a 2',3' cyclic phosphate because of the unfavourable orientation of the 2'-hydroxyl group. The study of arabinonucleotides might also provide useful information as to the importance of the sugar residue to the biological action of ribonucleotides. Although conformational studies of arabinoadenosine mono and dinucleotides have appeared [221, 222], the further comparison of the physical properties of arabino- and ribonucleotides, such as the phosphodiester backbone conformation and the base stacking properties, might eventually enable a better understanding of the basis for their widely different importance in biological systems.

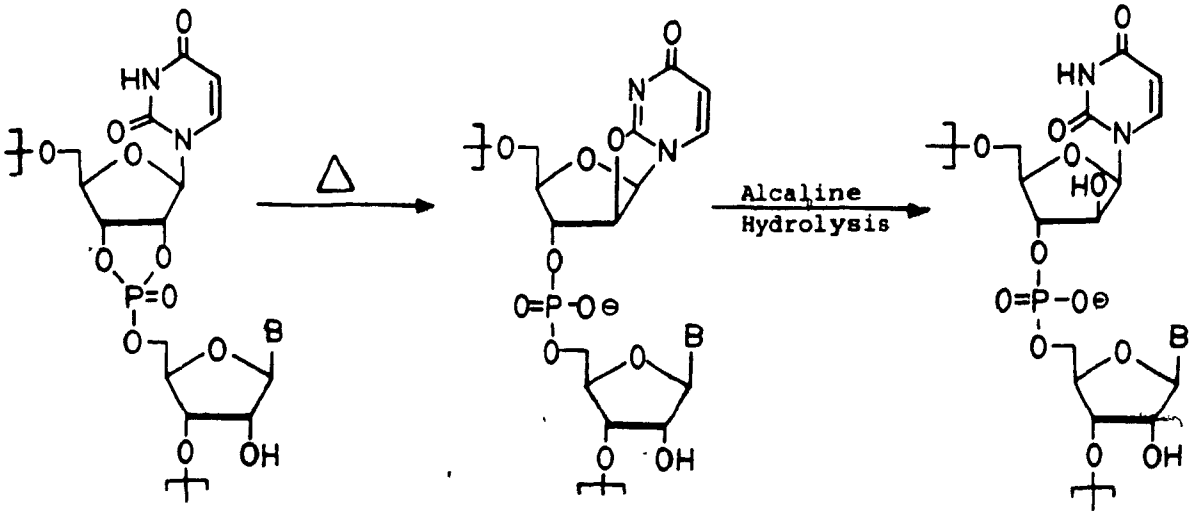
In a first step toward these goals, the preparation of derivatised araA nucleosides must be undertaken. The protection requirements of arabinonucleosides for their eventual incorporation into nucleotides resemble those of ribonucleosides because of the presence of the 2'-hydroxyl function. However, this position is less accessible to derivatisation in the arabino series due to its orientation toward the β face of the sugar ring. This face is more sterically crowded than the α face due to the base as well as the 5'-hydroxymethyl group. Various hydroxyl-derivatised arabinonucleosides have been reported in the literature [215, 223-228]. Also, a series of dinucleotides containing arabinocytidine have been prepared by Wechter [229] but as no selective protection was employed for the hydroxyl groups in the 2' and 3' positions, the condensations resulted in mixtures of 2',5' and 3',5' isomers which proved difficult to separate. The preparation of mixed ribo and arabino di- and trinucleotides has also been reported [230, 231]. In these procedures, a 2',3'-dibenzoyl or di-t-butyldimethylsilylarabinonucleoside was introduced as the 3'-terminal residue

with no further chain extension.

Scheme XXIII



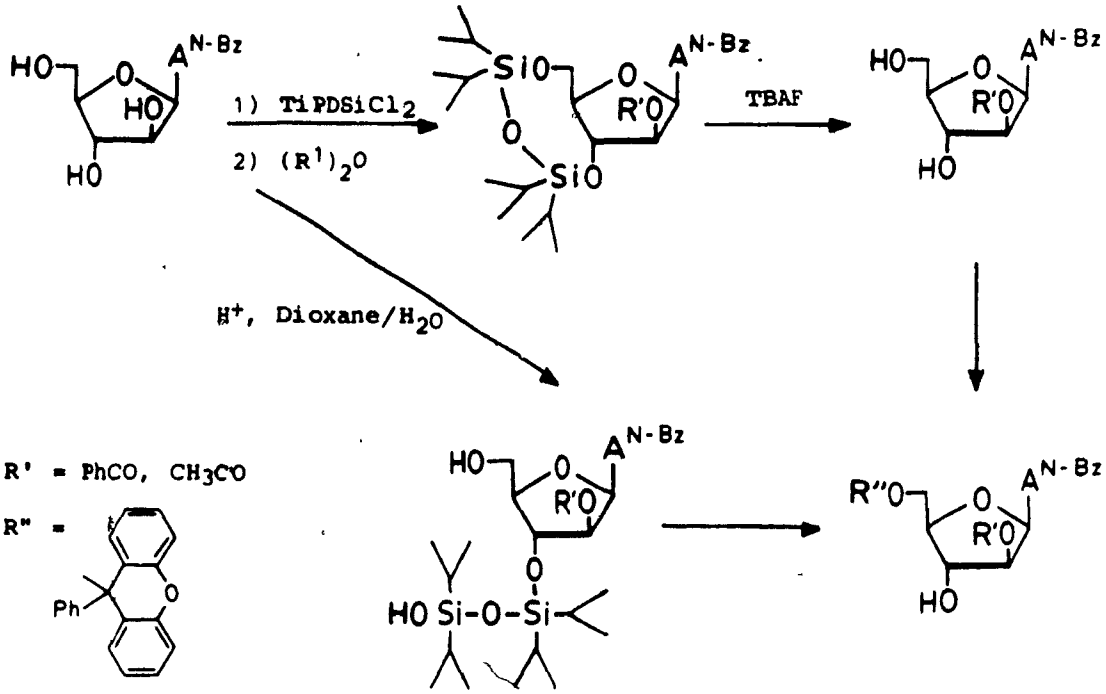
Scheme XXIV



Another approach to the incorporation of arabinonucleosides into nucleotides proposed by Ogilvie et al.[232] involves the coupling of a 2',3' protected ribonucleoside to a 5'-tritylated-2'-phosphorylated-$O^{2'}$-2'-anhydronucleoside which is later hydrolysed to the arabinonucleoside by treatment with ammonium hydroxide (Scheme XXIII). Thermal rearrangement

via labile cyclic triester intermediates of a polyuridylnucleotide to a randomly heteropolymeric arabino/ribonucleotide has also been reported (Scheme XXIV) [233]. However, both these procedures are limited to nucleosides which possess a C-2 keto function (pyrimidines) as they require formation of an anhydro intermediate. Neither method is applicable to the preparation of araA nucleotides.

Scheme XXV



At the time this work was undertaken there were, to our knowledge, no reports of araA nucleosides selectively derivatised in the 2' and 3' positions for incorporation into a polynucleotide chain. More recently, two independant groups reported the preparation of araA tri- and tetranucleotides using the solution phosphotriester approach [229, 234, 235]. In both syntheses, the 3' and 5' positions of N-6 benzylated araA nucleosides were simultaneously silylated by treatment with 1,3-dichloro-1,1,3,3-

tetraisopropyldisiloxane thus differentiating the 2'- and 3'-hydroxyls (Scheme XXV). Following protection of the 2'-hydroxyl, the 5' position is deprotected either selectively or along with the 3' position and an acid labile group (monomethoxytrityl or pixyl) is then introduced. In both these procedures, the preparation of a suitably 2' and 5' protected arabinonucleoside requires at least four steps.

As part of this laboratory's overall objective of preparing suitably protected arabinonucleosides, the techniques developed for the protection of ribonucleosides [123, 236] and successfully applied to their incorporation into ribonucleotides [137-239] have been introduced to the derivatisation of arabinoadenosine. Preliminary results for the preparation of araA derivatives [240] and incorporation into dinucleotides [241] have been obtained in this laboratory. The preparation of araA derivatives has been completed and these have been fully characterised. The protection of the 5' position of araA with the DMT group was undertaken as well as selective blocking of the 2'- and 3'-hydroxyl positions with the TBDMS group by the intermediary of suitable catalysts.

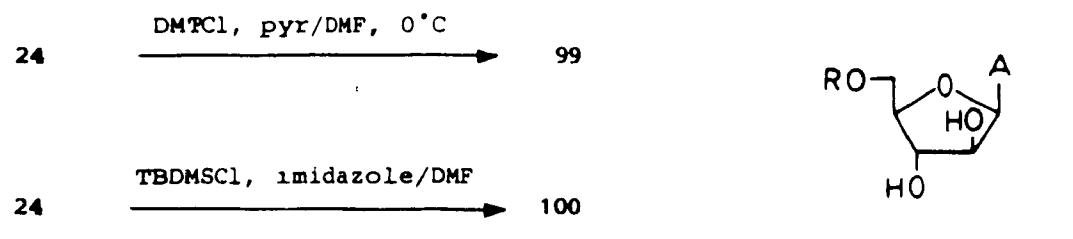
2.1 Synthesis of the Protected Arabinoadenosine Nucleosides

2.1.1 Dimethoxytritylation of Arabinoadenosine

The tritylation of araA in the 5' position, represented in Scheme XXVI, was effected using a modification of a procedure developed by Khorana et al. [90, 242]. The original report described the reaction of a deoxynucleoside with dimethoxytrityl chloride in pyridine at room temperature.

When applying this technique to the protection of ribonucleosides, Ogilvie and co-workers found that despite the greater reactivity of the 5'-hydroxyl, several side products were formed notably ditritylated (2',5'- and 3',5'- and N-tritylated material) and an improvement of the procedure was reported [236]. The formation of undesired side products, such as N-6 tritylated araA, was minimised by adding the dimethoxytrityl chloride portionwise over a 9 hour period to a solution of the nucleoside in pyridine/DMF maintained at 0°C. This modification was applied to the tritylation of araA (**24**). The major product observed by TLC was the desired 5'-dimethoxytritylaraA (**99**). Tritylation of the less reactive exocyclic amine function and of the less accessible secondary hydroxyl groups was minimal and little unreacted starting material was recovered. Following precipitation of the reaction mixture from ice water, compound **99** was isolated as a white powder in 70% yield. The UV absorption maximum at 259 nm (Table X) confirms that substitution has not occurred at the base. The physical properties and yields of all derivatised araA nucleosides prepared are listed in Table X; characterisation of the products by ^{1}H and ^{13}C NMR is discussed in section 2.2.

Scheme XXVI



99 **100**

R = **DMT** **TBDMS**

TABLE X

Physical Properties of Protected Arabinoadenosine Nucleosides

Product	Preparation	Yield (%)	Isomer Ratio 2'/3'	Melting Point (°C)	λ_{max} nm (EtOH)	λ_{min} nm (EtOH)	R_f
5'DMT (99)	DMTCl DMF/pyr	70	---	139-140	259 232	251 222	0.44 b 0.61 c
5'Si (100)	DMF/imid.	65	---	155-156[1]	258	228[1]	0.15 a 0.38 b
2',5'Si[2] (101)	$AgNO_3$/pyr THF	93	30/1	189	258	232	0.64 a 0.86 b
3',5'Si (102)	$AgNO_3$/MPNO THF	41	1/2	174-175	257	227	0.51 a 0.71 b
5'DMT-2'Si (103)	$AgNO_3$/pyr THF	52	2/1	116-117	257 234	252 223	0.60 a
5'DMT-3'Si (104)	$AgNO_3$/MPNO THF	49	1/2	110-111	258 234	250 225	0.46 a
2'Si (106)	AcOH, 80%	80	---	193-194	257	227	0.16 a 0.47 b
3'Si (107)	AcOH, 80%	77	---	200-201	258	228	0.21 a 0.52 b
2',3'Si[2] (108)	----	--	---	d 240	257	227	0.57 a

1) Literature melting point: 157-158°C; UV λ_{max} (MeOH) = 259 (ref. 228). 2) prepared by Dr. Hosein Hakimelahi (ref. 243). TLC solvents: a) CHCl3/DMF (8:2); b) CHCl3/EtOH (8:2); c) idem (7:3).

2.1.2 Preparation of t-Butyldimethylsilylated Arabinoadenosines

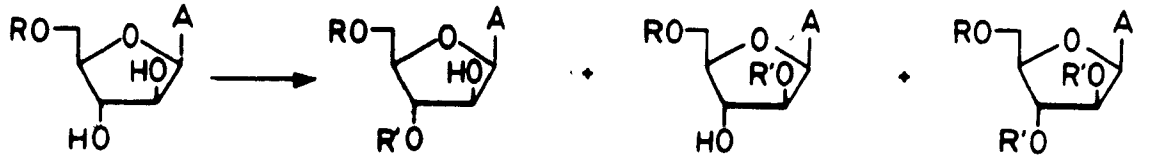
The first step undertaken was the preparation of 5'-t-butyldimethylsilylaraA (100) as earlier reported by Baker et al.[228] using the traditional procedure for the deoxy- and ribonucleoside silylations. AraA was treated with TBDMSCl in the presence of imidazole using DMF as solvent (Scheme XXVI).

When applied to 5'-protected ribonucleosides, the DMF/imidazole procedure yields a roughly equal proportion of 2'- and 3'-silylated isomers although the 2'-derivative is usually slightly favoured [123, 244]. In the arabino series, this method results in an isomeric ratio of approximately 6:1 in favour of the 3',5'-disilylated nucleoside. This is due to the greater steric hindrance at the 2' position caused by the base [241]. These isomers are generally difficult to separate and, because of the orientation of the 2'-hydroxyl group, the more abundant 3'-silyl is not easily isomerised to the desired 2' derivative [241] unlike the equivalent ribose derivative [104, 123, 245]. As nucleotide chain extension requires a free 3'-hydroxyl group, efficient and selective 2' protection is desirable. Ogilvie et al.[236, 246] have developed a series of catalytic systems which favour either 2' or 3' silylation in ribonucleosides and the preliminary applications of similar systems to arabinonucleosides have proved successful [240].

The procedure used for selective 2' or 3' silylation of araA involves the formation of a t-butyldimethylsilyl-base complex which is believed to be the silylating agent involved. This is achieved by adding the nucleoside to a silver nitrate, TBDMSCl and base suspension in THF previously stirred at room temperature for 15 to 60 minutes. Reaction occurs selectively at the 2' site when pyridine is used as base whereas the 3'-silyl isomer is favoured when 3-methylpyridine-N-oxide (MPNO) is used (Table X). In the preparation of the disilylated nucleosides (101, 102) (Scheme XXVII), 2.2 to 2.6 moles of silyl chloride per mole of nucleoside were used. This was decreased to 1.5 to 1.7 when selectively silylating 5'-DMTaraA (103, 104). Due to the acid sensitivity of the DMT group, the work-up procedures of these reactions require immediate treatment with a

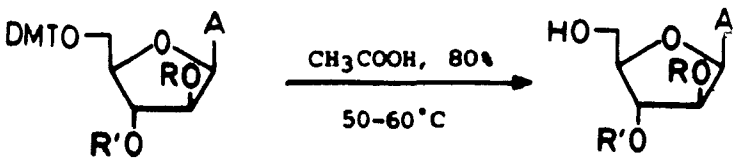
sodium bicarbonate solution (5%). The 2'- and 3'-silylararibonucleoside isomers are difficult to separate often requiring repeated purifications by chromatography. In addition, the derivatives prepared (103, 104) are susceptible to detritylation during chromatography on silica gel. This problem was minimised by the addition of traces of triethylamine to the elution solvents. The 2',3'-disilyl-5-DMTaraA (105) was prepared by the DMF/imidazole route with 4 moles of TBDMSCl per mole of DMTaraA (99). Compounds 101 and 108 were prepared in this laboratory by Dr. Hakimelahi [243]. All compounds were purified by chromatography and isolated as white powders.

Scheme XXVII



	R		R	R'		R	R'		R	R'
24	H	101	TBDMS	TBDMS	102	TBDMS	TBDMS	105	DMT	TBDMS
99	DMT	103	DMT	TBDMS	104	DMT	TBDMS			

Scheme XXVIII



	R	R'
103, 106	TBDMS	H
104, 107	H	TBDMS
105, 108	TBDMS	TBDMS

103, 104, 105 106, 107, 108

The tritylated analogues (103, 104, 105) upon treatment with 80% acetic acid at 50-60°C afforded the corresponding 5'-hydroxyl derivatives (106,

107, 108) as white powders in very good yields (77-80%), (Scheme XXVIII).

2.2 ^{13}C and ^{1}H NMR Characterisation of the Arabinoadenosine Derivatives

The consistency of the UV absorption maxima (257-259 nm) having established the absence of base substitution, the prepared compounds were fully characterised by ^{13}C and by ^{1}H NMR. The number of dimethoxytrityl and t-butyldimethylsilyl groups was easily determined by the relative integrations of the TBDMS proton signals (-0.3 to 1.1 ppm) and the DMT methoxy signals (3.9 ppm) to those of the nucleoside base or sugar ring protons. While monosilylation or tritylation invariably occurs at the more reactive primary 5'-hydroxyl group, silylation of a 5'- protected arabinonucleoside leads to a mixture of 2'- and 3'-isomers. Unequivocal determination of the position at which substitution has occurred can be achieved by ^{13}C NMR chemical shift differences and to a lesser extent by ^{1}H NMR peak positions.

2.2.1 ^{13}C NMR Characterisation of the Protected Arabinoadenosines

The ^{13}C NMR chemical shifts of the protected araA derivatives are listed in Table XI. These have been unambiguously assigned by ^{1}H-^{13}C heteronuclear decoupling. In a series of experiments, the H-1' or H-2' signals of the protected nucleosides were selectively irradiated to determine the corresponding carbon signals which showed the absence of proton splitting and an NOE enhancement. Selective decoupling experiments were not performed for the assignment of the C-2' and C-3' signals of 2'- and 3'-TBDMSaraA (106, 107) as detritylation of 103 and 104 lead, in each

case, to the formation of a single product. As no isomeric mixture is produced, C-2' and C-3' signals are easily assigned by the known C_α deshielding caused by a silyl group. The araA (24) ^{13}C peak positions were assigned according to those reported by Robins et al.[247]

TABLE XI

^{13}C Chemical Shifts of the Protected Arabinoadenosine Nucleosides

	araA	5'DMT	5'Si	2;5'Si	3;5'Si	DMT2'Si	DMT3'Si	2'Si	3'Si	2;3'Si
C	(24)	(99)	(100)	(101)	(102)	(103)	(104)	(106)	(107)	(108)
6	155.91	155.99	155.97	155.86	155.92	155.97	156.02	155.91	155.87	155.85
2	152.43	152.51	152.52	152.47	152.52	152.63	152.63	152.25	152.43	152.54
4	149.40	149.53	149.55	149.15	149.44	149.12	149.55	149.19	149.32	149.06
8	140.30	139.91	140.06	139.40	140.00	139.68	140.27	139.89	140.23	139.85
5	118.25	118.27	118.26	118.28	118.15	118.37	118.32	119.55	118.14	118.28
1'	83.57	83.46	83.14	83.33	83.25	84.06	83.09	83.51	83.51	83.66
4'	84.07	82.34	83.14	83.36	83.25	83.36	81.96	84.13	84.29	85.05
2'	75.67	75.47	75.70	77.02	75.64	76.67	75.70	77.09	75.75	76.86
3'	75.00	75.62	74.40	74.86	76.02	76.44	76.94	74.96	76.55	76.71
5'	60.87	63.69	62.54	62.47	61.94	64.32	63.24	60.77	60.48	60.63
$SiC\underline{C}H_3$			25.85	25.80	25.74	25.20	25.58	25.27	25.87	25.66
				25.20	25.64			25.00	25.69	25.29
					25.10			24.94		
$Si\underline{C}CH3$			18.14	18.06	17.98	17.22	17.60	17.22	17.71	17.59
				17.31	17.65					17.22
$SiCH_3$			-5.33	-5.21	-4.47	-5.44	-4.47	-5.22	-4.49	-4.54
				-5.38	-5.06	-5.98	-5.11	-5.98	-4.85	-4.66
				-5.84	-5.49					-5.31
										-5.74

Spectra recorded in dmso-d6 (δ = 39.5) on a Varian XL-200 instrument. δ reported in ppm relative to TMS.

From the data presented in Table XII, two principal effects are seen to result from substitution of araA. The presence of a TBDMS group causes deshielding of C_α by 1.1-1.7 ppm and substitution by a DMT group causes deshielding by ca 3 ppm relative to the corresponding signal in araA. The signals of sugar ring carbons other than C_α generally remain unaffected

with the exception of C-4' which is shielded in all 5' substituted analogues (0.7-1.7 ppm). This effect is particularly important in the 5'-DMT-3'-TBDMS derivative (2.11 ppm) possibly reflecting the steric crowding felt at the C-4' position. Another exception is the deshielding of C-3' (1.4 ppm) in the 5'-DMT-2'-TBDMSaraA. Curiously, the downfield shift of the C-3' signal relative to araA is greater in this compound than that of the silylated C-2' signal (1.0 ppm). The deshielding of C-3' might be the result of an anisotropic effect of the DMT group aromatic rings. While the proportion of C-3' deshielding due to such effects cannot be estimated in the 5'-DMT-3'-TBDMS compound (this peak position is shifted downfield due to silylation), it is negligible in the 5'-DMT derivative (0.6 ppm). Thus, it is possible the presence of the 2' substituent causes the sugar ring to adopt a conformation which entails a greater interaction between the 5'-DMT group and C-3'.

TABLE XII

$\Delta\delta$ of ^{13}C of the Protected Arabinoadenosine Analogues Relative to Arabinoadenosine

	C-6	C-2	C-4	C-8	C-5	C-1'	C-4'	C-2'	C-3'	C-5'
5'Si	0.06	0.09	0.15	-0.24	0.01	-0.93	-0.43	0.03	-0.60	1.67
2'Si	0.00	-0.18	0.09	-0.41	1.31	0.06	-0.06	1.42	-0.04	-0.10
3'Si	-0.04	0.00	-0.08	-0.07	-0.11	0.22	-0.06	0.06	1.55	-0.19
2',5'Si	-0.05	0.04	-0.25	-0.90	0.03	-0.71	-0.24	1.35	-0.14	1.60
3',5'Si	0.01	0.09	0.04	-0.30	-0.10	-0.82	-0.32	-0.03	1.02	1.07
2',3'Si	-0.06	0.11	-0.34	-0.45	0.03	0.98	0.09	1.19	1.71	-0.24
5'DMT	0.09	0.08	0.13	-0.39	0.02	-0.73	-0.61	0.05	-0.82	3.82
DMT-2'Si	0.06	0.20	-0.28	-0.62	0.12	0.04	-0.21	0.84	1.78	3.45
DMT-3'Si	0.11	0.20	0.15	-0.03	0.07	-0.98	-1.61	0.03	1.94	2.37

$\Delta\delta$ reported in ppm. (-) denotes an upfield shift relative to araA.

In the analogues prepared, the substitution of sugar ring hydroxyl functions have little effect on the base carbon signals other than a slight shielding of C-8 when the 2' or 5' positions are protected. The implications of this shielding are further discussed in section 2.2.2. Of the TBDMS carbon signals, the t-butyl are farthest downfield. The quarternary carbon signals appear upfield of the t-butyl and the dimethyl are the most shielded appearing upfield of TMS.

2.2.2 ^{1}H NMR Analysis of the Protected Arabinoadenosines

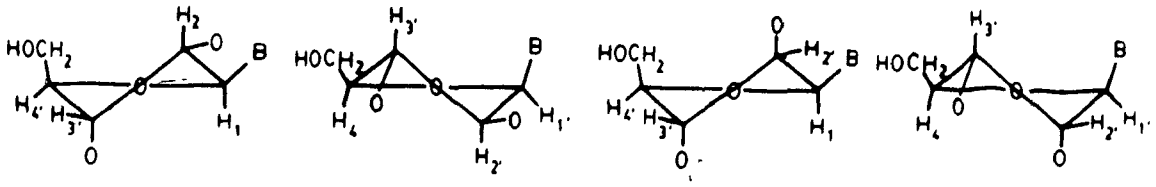
^{1}H NMR chemical shifts of the protected arabinoadenosine nucleosides are listed in Table XIII. Assignments of araA (**24**) protons were made according to those determined by Robins et al.[247]. The differentiation of the H-2' and H-3' peaks were determined by homonuclear decoupling of the H-1' signal for all compounds prepared.

It is apparent from the ^{1}H chemical shifts reported that the most important effects induced by substitution of a sugar ring hydroxyl group of araA are a deshielding (0.1-0.2 ppm) of the corresponding proton in the case of silylation and a shielding (0.4 ppm) for tritylation. Other small but consistent shift differences can be useful for the identification of araA derivatives notably in distinguishing between 2'- and 3'-silyl isomers. As has been reported for araC [241], the H-1' chemical shifts of 3'-silyl isomers are consistently upfield (.04-.08 ppm) from those of 2'-isomers due to the interaction between H-1' and the 3'-silyl group. The effect is larger in 5'-tritylated derivatives because of increased steric crowding. The opposite effect, i.e. greater shielding of H-1' in 2'-silyl isomers, was reported in cytidine derivatives [245]. This effect is the result of the greater proximity between the OSi-2' and H-1' in ribonucleo-

sides than arabinonucleosides. As both groups are oriented toward the αface of the ribose sugar ring, 2'-silylation has a greater shielding effect on H-1' than 3'-silylation. In some instances, $J_{H1',H2'}$ differences serve to distinguish silyl isomers in the ribose series [248] but these are not useful for arabinonucleoside characterisation (Table XIII). The constant varies with the dihedral angle between coupled protons and is thus dependant upon ring conformation. The conformation of 2' and 3' derivatised adenosines have been studied for several substituents and solvents [249]. It has been determined that, in general, the N conformation, for which $J_{1',2'}$ values are smaller, is preferred for 3' substituted adenosine. Conversely, the S form for which $J_{1',2'}$ values are larger, is predominant for 2' substituted adenosine (Figure 11). This seems to indi-

Figure 11

The S and N Conformations of the Ribose and Arabinose Rings



Ribose Arabinose

cate that a substituent in the 2' or 3' position prefers a pseudo-equatorial orientation to minimise unfavourable steric effects. In araA, the variations in the dihedral angle between H-1' and H-2' in going from the N to the S form are very small. However, $J_{2',3'}$ and $J_{3',4'}$ values have been used to confirm that the N conformation, in which both the 2'- and 3'-hydroxyl groups are in a pseudo-equatorial orientation, is preferred for

araA in aqueous solution [250]. The N conformation is also predominant for 2' and 3' derivatised arabinoadenosines for a great number of substituents and solvents [251, 252].

TABLE XIII

^{1}H Chemical Shifts of the Protected Arabinoadenosine Nucleosides

	araA	5'DMT	5'Si	2'5'Si	3'5'Si	DMT2'Si	DMT3'Si	2'Si	3'Si	2'3'Si
H	24[a]	99[a]	100[a]	101[b]	102[b]	103[b]	104[b]	106[a]	107[a]	108[a]
2	8.32	8.30	8.33	8.31	8.30	8.32	8.32	8.30	8.32	8.31
8	8.29	8.26	8.30	8.23	8.29	8.15	8.30	8.27	8.29	8.22
NH2	6.68	6.74	6.82	6.70	6.75	6.75	6.86	6.71	6.72	6.71
1'	6.54	6.58	6.54	6.57	6.52	6.64	6.56	6.56	6.51	6.56
J*	(4.6)	(4.2)	(4.5)	(5.0)	(4.6)	(4.3)	(4.9)	(4.8)	(4.5)	(4.0)
3'	4.55	4.50	4.51	4.52	4.60	4.40	4.68	4.58	4.66	4.63
2'	4.38	4.50	4.15	4.58	4.46	4.46	4.50	4.59	4.39	4.46
4'	4.16	4.25	4.10	4.10	4.08	4.36	4.14	4.09	4.10	4.12
5'	4.00	3.62	4.10	4.10	4.08	3.68	3.58	3.97	3.97	3.99
		3.47				3.44	3.51			
OCH3		3.90				3.91	3.90			
SiC_{CH3}			1.05	1.06	1.08	0.73	1.00	0.76	0.98	1.09
				0.79	1.06					0.82
SiCH3			0.24	0.12	0.12	0.12	0.27	0.13	0.31	0.33
						0.02	0.19	-0.17		0.13
										-0.27

Spectra recorded in acetone-d6 (δ = 2.17) on a) a Varian XL-200; b) a Brüker 400 MHz instrument. δ reported in ppm relative to TMS. (*) $J_{1',2'}$ reported in Hz.

In comparison to araA signals, shielding of H-4' is apparent for all silylated derivatives. However, important H-2' shielding (0.23 ppm) occurs only in the 5'-TBDMS derivative (100) (Table XIVa). 5'-Tritylation causes slight deshielding of the α face protons (H-2', H-4' and H-1') and shielding of H-3'. In the 5'-DMT-3'-TBDMS derivative (104), the net effect on H-2' is a shielding caused by the 3'-silyl group. Another interesting effect is the deshielding of H-2' upon 3'-silylation of 5'-TBDMSaraA

(Table XIVb). Silylation of 5'-DMTaraA (99) in the 2' position causes shielding of both H-3' and deshielding of H-4' while only the latter is shielded upon 3'-silylation (Table XIVc).

TABLE XIV

$\Delta\delta$ ^{1}H of the Protected Arabinoadenosine Analogues

relative to	H-2	H-8	H-1'	H-3'	H-2'	H-4'	H-5'
a) araA							
5'TBDMS	0.01	0.01	0.00	-0.04	-0.23	-0.06	0.10
2'TBDMS	-0.02	-0.02	0.02	0.03	0.21	-0.07	-0.03
3'TBDMS	-0.00	0.00	-0.03	0.11	0.01	-0.06	-0.03
2',5'TBDMS	-0.01	-0.06	0.03	-0.03	0.20	-0.06	0.10
3',5'TBDMS	-0.02	0.00	-0.02	0.05	0.08	-0.08	0.08
2',3'TBDMS	-0.01	-0.07	0.02	0.08	0.08	-0.04	-0.01
5'DMT	-0.02	-0.03	0.04	-0.05	0.12	0.09	-0.38
5'DMT-2'TBDMS	-0.00	-0.14	0.10	-0.15	0.08	0.20	-0.44
5'DMT-3'TBDMS	0.00	0.01	0.02	0.13	0.12	-0.02	-0.45
b) 5'TBDMSaraA							
2',5'TBDMS	-0.02	-0.07	0.03	0.01	0.43	0.00	0.00
3',5'TBDMS	-0.03	-0.01	-0.02	0.09	0.31	-0.02	-0.02
c) 5'DMTaraA							
5'DMT-2'TBDMS	0.02	-0.12	0.05	-0.10	-0.04	0.11	0.02
5'DMT-3'TBDMS	0.02	0.04	-0.03	0.19	0.00	-0.11	-0.04
d) 2'TBDMSaraA							
2',5'TBDMS	0.01	-0.04	0.01	-0.06	-0.01	0.01	0.13
2',3'TBDMS	0.01	-0.05	0.00	0.05	-0.13	0.03	0.02
5'DMT-2'TBDMS	0.02	-0.12	0.08	-0.18	-0.13	0.27	-0.41
e) 3'TBDMSaraA							
3',5'TBDMS	-0.02	0.00	0.01	-0.06	0.07	-0.02	0.11
2',3'TBDMS	-0.01	-0.06	0.05	-0.03	0.07	0.02	0.02
5'DMT-3'TBDMS	0.00	0.01	0.05	0.02	0.11	0.04	-0.43

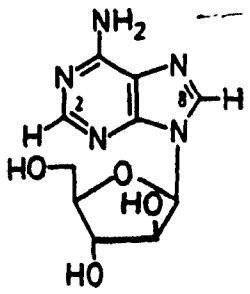
$\Delta\delta$ reported in ppm. (-) denotes an upfield shift relative to the parent compound.

The protons of the TBDMS groups possess different chemical shifts depending on the position of substitution (Table XIII). The t-butyl protons appear as a singlet at lower field for 5'-TBDMSaraA (1.00-1.08 ppm) and 3'-TBDMSaraA (0.98-1.06 ppm) and higher field for 2'-TBDMSaraA (0.73-0.82 ppm) because of the shielding effects of the base ring currents on a 2'-silyl group. The dimethylsilyl proton signals are also characteristic of the position of substitution in the araA derivatives. As in the adenosine series [123], the 3'-silyl signals are farthest downfield, followed by the 5'-silyl and 2'-silyl. The methyl protons of each silyl group appear as a single broadened peak for the 5'-silyl and 3'-silyl as well as for both the 2',5'- and 3',5'-disilylated araA isomers. The equivalence of the methyl groups implies there is very little rotation restriction about O-Si bonds in these molecules. These results are not consistent with those reported for derivatised araC in which the methyl signals appear at different positions [241]. Curiously, the two methyl signals of 2'-TBDMSaraA are resolved.

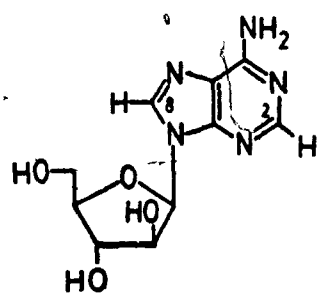
From the results presented in Table XIVa, it is also apparent that substitution of the sugar ring hydroxyl groups has remarkably little

Figure 12

The Syn and Anti Conformations about the Glycosidic Bond



Syn



Anti

effect on the H-2 base signal. This chemical shift varies within a range of ± 0.01 ppm regardless of the substitution pattern of the ribose ring. These effects indicate that the predominant conformation about the glycosidic bond is anti (Figure 12) and this is consistent with reported conformational studies of 2', 3' and 5' substituted arabinoadenosines [251].

Monosubstitution, whether in the 5', 2' or 3' position (**99, 100, 106, 107**), has very little discernible effect on the H-8 signal. The same is true for 3',5' substituted araA (**102, 104**). However, when both the 5' and 2' positions are derivatised (**101, 103**), there is a marked upfield shift of the H-8 signal reflecting increased steric crowding of this position. A comparison of the H-8 signals of the 2',5'-disubstituted compounds and 2'-TBDMSaraA reveals a 0.04 ppm shielding upon addition of a 5'-silyl group and a 0.13 shielding when a 5'-trityl group is introduced. The difference in these effects can be attributed to the greater bulk of the latter function. The 2' and 5' substituents have a combined effect which is not apparent in 3',5'-isomers presumably because rotation around the glycosidic bond is more restricted in the former compounds and crowding interactions are not easily minimised. A smaller but significant shielding of H-8 is noted in the 2',3'-disilyl derivative possibly due to steric effects between the 3' substituent and the 5'-methylhydroxy group which force the latter toward the base residue.

2.3 Conclusion

Tritylation of the 5' position of araA was effected with minimal side reactions. Silylation in the 2' and 3' positions were performed selectively with silver nitrate/base catalytic systems. The preparation of araA nucleosides suitably protected for nucleotide synthesis was achieved in fewer steps by these procedures than by previously reported methods for selective 2' and 5' protection. Furthermore, the selectivity obtained facilitates the separation of 2'- and 3'-silyl derivatives which is more difficult when isomers are present in equal proportion. The 5'-DMT-2'-TBDMSaraA derivative prepared is suitable for incorporation by the 3'-hydroxyl position into a nucleotide chain. Moreover, this nucleoside is amenable to chain prolongation from the 5' position following cleavage of the acid labile trityl group.

Both ^{1}H and ^{13}C NMR serve to determine the position of silylation of araA as the effects of hydroxyl substitution on the corresponding carbon and proton signals are known. Silylation and tritylation induce deshielding of C_{α} by 1-2 ppm and 3-4 ppm respectively. Heteronuclear ^{13}C selective decoupling experiments are necessary for the initial identification of 2'- and 3'-silyl isomers. However, once the original assignments have been established, ^{1}H NMR has proved sufficient for isomer differentiation. In the 2'-TBDMS isomers, the H-8 signal is consistently upfield and the H-1' consistently downfield relative to their positions for 3'-isomers. However, the effect on H-8 is more pronounced in disubstituted (2',5'; 3',5') compounds.

In the ribonucleoside series, 2'-silyl isomers can be differentiated from the 3' derivatives by the slower TLC mobility of the latter on silica gel. This is also the case for all the disubstituted araA analogues prepared (Table XI) as confirmed by ^{13}C NMR. However, the trend is reversed for the 2'- and 3'-monosilyl araA derivatives although the R_f differences between these two compounds are small.

CHAPTER THREE

Application of ^{29}Si NMR to the Characterisation of Nucleosides

3.0 Introduction

Silyl ethers were introduced to synthetic chemistry for the protection of hydroxyl groups in the early 1970's. Since this time, organosilicon reagents have become increasingly popular in organic syntheses [253] and more particularly in the protection of nucleosides [102, 103]. The widespread use of silyl protecting groups, the advent of multinuclei spectrometers and the development of signal enhancing pulsing sequences have combined to render ^{29}Si NMR a viable technique for the characterisation of organic molecules and, possibly, of protected nucleosides and nucleotides.

The nuclei commonly observed for the study of nucleotides by NMR (^{1}H, ^{13}C, ^{31}P) are either present in high natural abundance or are sensitive enough to produce relatively strong signals. Silicon has one naturally occurring isotope with non zero spin ($\frac{1}{2}$), ^{29}Si, which is not very abundant (Table XV) and possesses a small gyromagnetic ratio (γ) [254]. This means there is a small energy difference between spin states hence small Boltzman population differences. To compensate for these effects and obtain an acceptable signal to noise ratio, ^{29}Si FT NMR experiments require a great number of acquisitions thus becoming so lengthy as to be impractical for routine investigations. In addition to these drawbacks, the gyromagnetic ratio is negative which causes the nuclear Overhauser effect enhancement (NOE) to be negative under proton decoupling condi-

TABLE XV

NMR Properties of Nuclei Useful for the Characterisation of Nucleosides and Nucleotides [255, 256]

Nucleus (Nu)	Spin	Natural Abundance (%)	Receptivity[a] Relative to ^{13}C	Usual Chemical[b] Shift Range (ppm)	$J_{Nu,H}$ range (Hz)
^{1}H	½	99.98	5.68×10^{3}	10	0-18
^{13}C	½	1.108	1.00	350	120-250
^{15}N	½	0.37	2.19×10^{-2}	1000	60-140
^{29}Si	½	4.70	2.09	400	147-420
^{31}P	½	100.00	377.00	710	37-1100

a) Receptivity = (abundance ratio [Nu/^{13}C]) x (sensitivity ratio [Nu/^{13}C])
b) δ 1H, 13C and 29Si usually reported relative to TMS; 15N usually relative to liquid NH_3 (25°C), $MeNO_2$ or NO_3^-; 31P, relative to 85% H_3PO_4

tions. Not only can this effect reduce signal intensity, it can give peaks a negative resulting intensity or can lead to signal cancellation. Moreover, the spin-lattice relaxation times (T_1) are generally long, exceeding 20 s for most ^{29}Si nuclei. To avoid saturation of the ^{29}Si nucleus, a delay period must be inserted after each pulse to allow time for relaxation. This further lengthens the time required for ^{29}Si NMR experiments.

Several means can be envisaged to circumvent the difficulties associated with the recording of silicon NMR spectra. ^{29}Si enriched experiments are one possibility though no such work has as yet been reported. Smaller pulse angles could be applied to decrease the time needed for relaxation but this would also decrease signal intensity. Paramagnetic relaxation reagents as well as gated decoupling experiments have been used in the past to eliminate the NOE but the signal to noise ratio remains unfavourable due to the low abundance of ^{29}Si and its small gyromagnetic ratio. More recently, the development of signal enhancing pulse sequences

has facilitated the study of insensitive nuclei such as ^{15}N and ^{29}Si. The INEPT experiment (Insensitive Nuclei Enhancement by Polarisation Transfer) introduced in 1979 by Morris and Freeman [257] has been successfully applied to the study of ^{29}Si NMR [258-261]. This experiment consists of a multipulse sequence in which the nuclear spin polarisation is transferred from the nucleus with the large Boltzman population difference, usually ^{1}H, to the species under investigation (Appendix, section II). Mathematical expressions have been derived by Doddrell et al.[258] to determine the theoretical signal enhancements which can be obtained by the INEPT experiment. ^{29}Si peaks show a five to ninefold increase relative to their intensities observed by the standard 2 pulse FT NMR sequence [258]. However, the efficiency of INEPT NMR is not only due to signal enhancement but also to the rapid rate at which the multipulse sequence may be repeated. In absence of polarisation, the pulse repetition rate depends on spin-lattice relaxation times (T_1 = 5 to 150 s for ^{29}Si [255]). In the INEPT experiment, the enhanced population difference is transferred from ^{1}H to ^{29}Si in each multipulse sequence therefore, only the ^{1}H nuclei need be allowed to relax (T_1 = 3 to 10 s for ^{1}H in aliphatic compounds). In addition to these advantages, ^{1}H coupled INEPT NMR allows a clearer distinction of multiplets as the enhancement of the weak outer lines of a multiplet is greater than that of the center lines.

Although the INEPT experiment has permitted the routine recording of ^{29}Si spectra, the method suffers from some limitations. The spin-spin relaxation times (T_2) must be long relative to the multipulse sequence as there must still be an observable ^{29}Si signal when acquisition is begun (at the end of the pulse sequence). Furthermore, the coupling constant ($J_{Si,H}$) must be resolvable by the spectrometer. Finally, it is imperative

that the correct $J_{Si,H}$ values for a given Si-H interaction and the exact number of coupled protons be determined. These figures are needed to calculate the delay times used in the multipulse sequence (Appendix, section II). Even small variations in delay time values can cause a considerable decrease of the signal enhancement factor.

^{29}Si chemical shifts cover a range of approximately 400 ppm from 60 to -350 ppm relative to TMS (a negative sign indicates an upfield peak position). However the practical range is reduced to ca 250 ppm when the low frequency tetraiodo derivative (SiI_4) is not included [254]. The wide dispersion of ^{29}Si shifts has proved helpful in the characterisation of organic molecules notably trimethylsilylated sugar derivatives [261, 262]. ^{29}Si chemical shifts are determined by a number of factors notably the electronegativity of substituents and the coordination number of the silicon atom (upfield shifts are observed for higher coordination numbers). Steric interactions as well as variations from a tetrahedral arrangement of substituents around silicon also influence ^{29}Si shifts. Furthermore, chemical shifts are affected by π bonding involving the d orbitals of silicon. The correlation between ^{29}Si peak positions and the above factors is not straightforward as no single factor is completely determinant. This is illustrated by the shifts listed in Table XVI. While ^{29}Si peak positions in going from $SiMe_4$ to SiH_4 do not seriously deviate from simple additivity of the substituent effects, this is not the case in going to $Si(OCH_3)_4$, possibly because OCH_3 is capable of backbonding. Although the theory of nuclear shielding is well developed, its application to the prediction and interpretation of ^{29}Si chemical shifts is problematic. Correlations between ^{29}Si peak positions and a number of factors (bond angles, lengths and strengths as well as π-orbital hybrid-

TABLE XVI

The Effect of Direct Substitution on ^{29}Si Chemical Shifts [256] for Compounds of the Type SiX_nY_{4-n}

Y	n = 1	n = 2	n = 3	n = 4	X
CH_3	-15.5	-37.3	-65.2	-93.1	H
CH_3	17.2	-2.5	-41.4	-79.2	OCH_3

Chemical shifts (δ) are reported in ppm.

isation) have been reported [254, 263, 264]. The more reliable predictions are based on substituent electronegativity. Ernst et al.[265] have found a relationship between δ_{Si} and Hammett σ_p values (representative of the sum of inductive and resonance effects) in arylsilanes ($ArSiY_3$). This approach has been recently improved by Janes and Oldfield [264] who have established a U shaped correlation between ^{29}Si δ and the electronegativity of ligands. This concurs with the similarity of chemical shifts observed for such different species as disilane (Si_2H_6, δ = -104.8 ppm) and quartz (SiO_2, δ = -107.4 ppm). In order to adhere to the correlation, silicon atoms must be divided into three categories: type "P" atoms bear substituents with lone pair electrons (capable of [d-p] π-bonding), type "S" bear ligands with only σ-bonding electrons and type "M" silicon bear a combination of ligands [264]. However, the mathematically determined trends still do not coincide completely with observed shifts and the justification of ^{29}Si peak positions remain purely qualitative for most compounds.

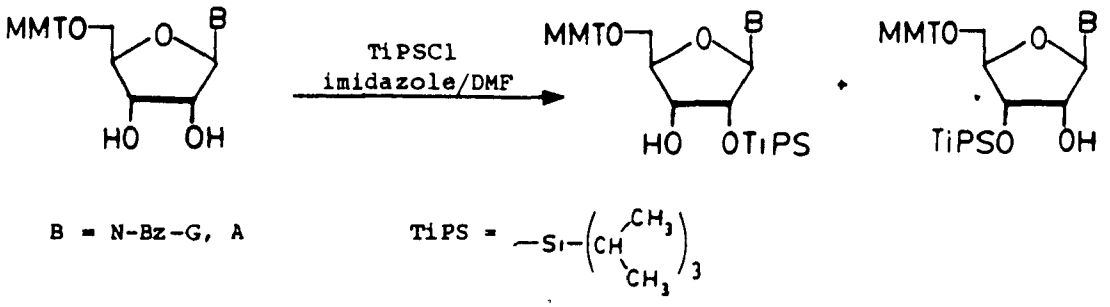
The characterisation of nucleosides and nucleotides by ^1H and ^{13}C NMR has now become quite sophisticated. The data garnered by these techniques, such as chemical shifts and coupling constants, can be interpreted on the basis of previous NMR studies in order to obtain information on aspects

ranging from preferred conformations to functionalisation positions. ^{13}C NMR presents some advantages over ^{1}H NMR in the characterisation of these molecules as decoupled ^{13}C spectra are generally more simple and allow study of the carbon skeleton of nucleosides and nucleotides. However, even ^{13}C NMR spectra can be so complicated as to be impractical for the characterisation of oligonucleotides of 5 units or more. Just as the advent ^{31}P NMR has proved invaluable for the characterisation of the phosphorous backbone of certain oligonucleotides [266], ^{15}N and ^{29}Si NMR might facilitate the study of nucleotide base residues and hydroxyl-protected sugar moieties. While the use of ^{15}N has been investigated [267-269], no reports have as yet been forthcoming concerning the usefulness of ^{29}Si NMR. As a first step toward such applications, the study of 5-trityl-2'-silylated ribonucleosides, the building blocks for nucleotide synthesis, was undertaken as well as that of several other silylated nucleoside derivatives.

3.1 Preparation of the Nucleoside Derivatives

The tritylated (MMT, DMT) and silylated (TBDMS) deoxyribo- and ribonucleosides were prepared according to procedures previously developed in this laboratory [123, 236, 244, 246]. In the preparation of the guanosine (G) residue suitably protected for nucleotide synthesis, the triisopropylsilyl group (TiPS) was selected in preference to the TBDMS group because of the ease of separation of 5'-trityl-2'-TiPS and 3'-TiPS isomers. These derivatives as well as those of adenosine (A) were prepared by treatment of N-Bz-5-MMTG or 5'-MMT-A with triisopropylsilyl chloride (TiPSCl) and imidazole in DMF (Scheme XXIX). The 2'-TiPS derivatives were obtained in

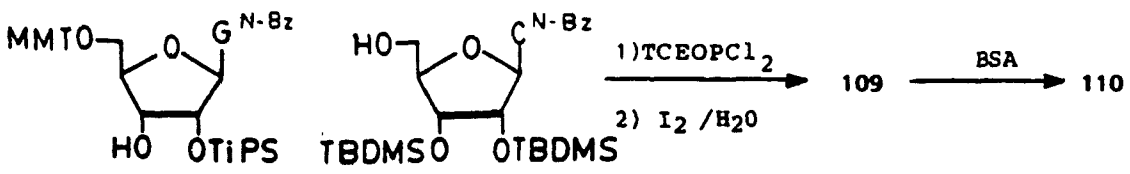
Scheme XXIX



B = N-Bz-G, A TiPS = $-Si-(CH(CH_3)_2)_3$

yields of 47% and 62% respectively. The separation of the guanosine 2' and 3' isomers was facilitated by adding traces of triethylamine to the elution solvents during chromatography on silica gel. Triethylamine is thought to eliminate possible intramolecular hydrogen bonding between the N-benzoyl amide oxygen and the 2'-hydroxyl proton in the 3'-TiPS guanosine derivative. In the absence of such H-bonding, the 3' isomer should become more polar and migrate more slowly on silica gel. Hence, the separation of this compound from the faster moving 2'-TiPS isomer should be facilitated. The di-TiPS derivatives were also prepared by the DMF/imidazole method [103]. The protection of arabinoadenosine is described in Chapter 2 and the arabinocytidine derivatives were prepared by N. Usman. The GpC dinucleotide (109) (Structure page 114) was prepared in 65% yield by condensation of N-Bz-5'-MMT-2'-TiPS-G with N-Bz-2',3'-diTBDMS-C in anhydrous THF using trichloroethylphosphodichloridite ($TCEOPCl_2$) in the presence of 2,4,6-trimethylpyridine. This was followed by treatment with 0.1 M solution of

Scheme XXX



benzenesulfonic acid (BSA) in acetonitrile affording the detritylated dinucleotide (110) (Structure page 114) in 90% yield.

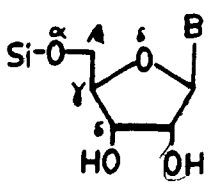
3.2 Characterisation of the Derivatives by ^{29}Si INEPT NMR

3.2.1 Selection of Parameters and Experimental Conditions

The transfer of polarisation from ^{1}H to ^{29}Si requires the existence of strong coupling between the two nuclei and is completely independant of the number of bonds separating them [266]. Thus, several possible Si-H interactions must be considered in the determination of $J_{Si,H}$. As previously mentioned, the choice of $J_{Si,H}$ is crucial to obtaining maximal signal enhancement in the INEPT experiment. The silicon atom of the TBDMS group can be coupled to both the methyl group protons as well as those of the t-butyl group whereas in the TiPS group, silicon is coupled to 3 α protons and 18 β protons. In addition, coupling may occur with sugar ring protons which are in the β, γ or δ positions relative to silicon (Figure 13). Variations in the magnitude of $J_{Si,H}$ with the number of atoms distancing the two nuclei are illustrated by the values listed in Table XVII.

Figure 13

Possible ^{1}H-^{29}Si Coupling Interactions in 5'-Silylated Ribonucleosides



Si = TBDMS, TiPS

TABLE XVII

$^{29}Si-^{1}H$ Coupling Constants [256]

^{1}J (Si-H)	^{2}J (Si-C-H)	^{3}J (Si-C-C-H)	^{4}J (Si-C-C-C-H)
147-420	1-13	1-8	1.0-1.5

$J_{Si,H}$ reported in Hz

Furthermore, the signal enhancement obtained with the INEPT pulse sequence is dependant on the number of coupled protons (n) [258]. It is therefore advantageous to select an average value of $J_{Si,H}$ in order to transfer polarisation from a maximal number of protons to the silicon atom. Values of $J_{Si,H}$ can, in principle, be determined by observing the ^{29}Si satellites in ^{1}H NMR spectra. However these satellites are very weak relative to the proton peaks of ^{28}Si isotopomers [256]. No such satellites are visible in the ^{1}H spectra of the nucleoside derivatives of interest in this study. Therefore, it was necessary to determine optimal INEPT parameters by a trial and error approach. A series of experiments were performed in which possible n values and a series of $J_{Si,H}$ were used in parameter determinations. The range of $J_{Si,H}$ was selected on the basis of coupling constants for similar compounds reported in the literature. The values leading to maximal signal enhancement were used in the INEPT NMR study of the protected nucleosides.

^{29}Si chemical shifts reported in the literature have not always been completely reliable [256]. There are instances in early papers of differing shifts observed for a single compound when studied under identical conditions. ^{29}Si peak positions are known to vary with the solvent electron pair donor ability as well as sample concentration. Furthermore, the peak position of reference compounds such as TMS have shown solvent dependen-

cies [270, 271]. Inconsistencies apparent in earlier studies can be attributed to instrument error, the high concentrations required for ^{29}Si NMR before the development of signal enhancing techniques as well as the limitations of the techniques used [256]. In the INEPT studies reported here, chemical shift variations of up to 2 ppm were found when observing one derivative at different concentrations in the same solvent. In order to avoid such errors, samples were prepared at concentrations of 15 to 30 mg/mL, a range for which variations in the ^{29}Si peak positions remain within acceptable limits (ca ±0.1 ppm). However, although this margin of error is quoted to ensure reproducibility of the observed shifts, the actual resolution of signals within a given sample is much greater. This is due to the digital resolution attained by the instrument under the experimental conditions used (0.03 ppm) and the sharpness of the observed ^{29}Si peaks.

3.2.2 Characterisation of 5' Tritylated 2'- and 3'-Silyl Ribonucleosides

The ^{29}Si INEPT spectrum of N-Bz-5'-MMT-2'-TiPS-G is reproduced in Figure 14 and the chemical shifts of the four common ribonucleosides suitably protected for nucleotide synthesis are listed in Table XVIII. Although remote substituents are known to have a lesser influence than those in the α position on ^{29}Si chemical shifts, the effect of a β, γ or δ substituent can be important. Furthermore, ^{29}Si shifts are considerably more sensitive to remote substituents which exert their influence through oxygen, as in the nucleoside derivatives, rather than carbon [272]. It is apparent that ^{29}Si shifts for nucleosides silylated in the 2' position are consistently downfield from those of the 3' protected derivatives by 0.7 to 1.5 ppm. This observation concurs with previous reports on the

Figure 14

^{29}Si INEPT Spectrum of N-Benzoyl-5'-Monomethoxytrityl-2'-Triisopropylsilylguanosine

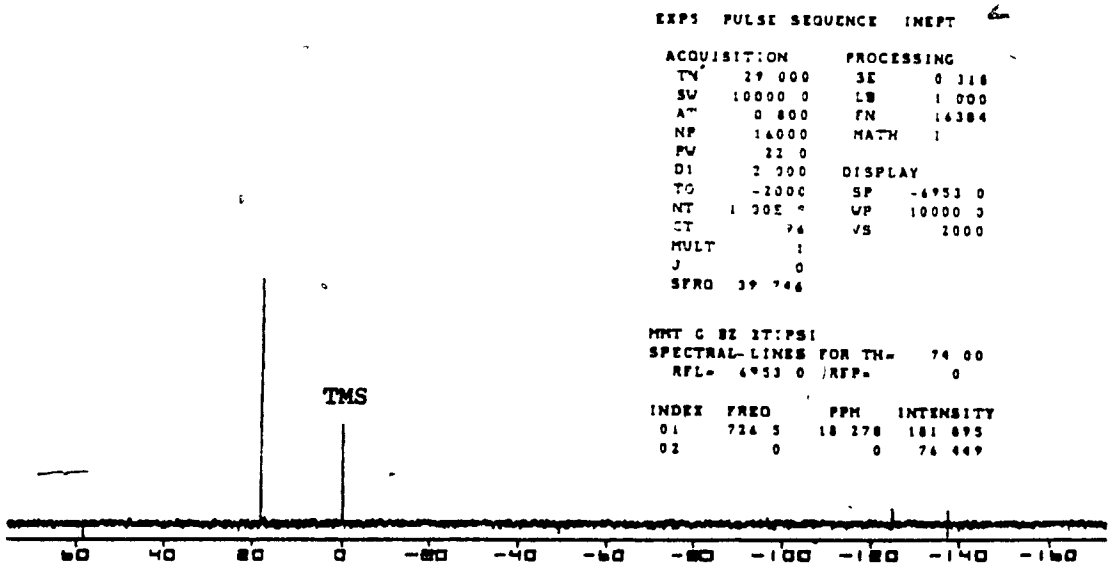


TABLE XVIII

^{29}Si Chemical Shifts of 5' Tritylated 2' and 3' Silylated Ribonucleosides

Product	2'silyl	3'silyl	2'3'disilyl
5'-MMT-TBDMS-U	25.7 (24.4)	24.6 (23.0)	
N-Bz-5'-MMT-TBDMS-C	25.6 (23.8)	24.1 (22.4)	21.8, 21.5 (21.8, 21.6)
5'-MMT-TBDMS-A	25.4	24.3	21.8, 20.8
5'-MMT-TiPS-A	18.2	17.5	
N-Bz-5'-MMT-TBDMS-A	25.6	24.7	22.4, 21.5
N-Bz-5'-MMT-TiPS-G	18.3	17.6	

δ reported in ppm relative to TMS. Spectra recorded in CDCl3; values in parentheses determined in CD3OD.

effects of electronegative δ substituents on ^{29}Si peak positions [273]. In compounds of the type Si-Y-CH_2CH_2-X, in which Y is capable of backbonding, the ^{29}Si signal is shifted downfield when the δ substituent (X) is changed from H to an electronegative group [272-274]. The opposite effect has been reported for compounds of the type R-Y-Si-CH_2-X. Possible explanations have been proposed for these observations [273]. In the former case, it may be that the electronegative substituent (X) decreases Y-Si backbonding resulting in a deshielding effect. Alternatively, in the latter compounds, an electronegative X substituent may favour Y-Si backbonding thus causing a net shielding effect on ^{29}Si. In the protected nucleosides, which resemble Si-Y-CH_2CH_2-X compounds, a 2'-silyl group is in the δ position relative to three electronegative substituents (the cyclic oxygen atom, the 3'-hydroxyl group and the N-7 or N-1 of the base). A 3'-silyl group is δ to only two electronegative substituents (the ribose ring oxygen atom and the 2'-hydroxyl group) which might account for the smaller chemical shifts observed. It is interesting to note that while ^{29}Si peak positions for 2' isomers are consistently downfield of the 3', no such generalisation can be made for the peak positions of the silyl group protons and carbons. For 5'-MMT-U, the ^1H and ^{13}C shifts of the 2'-silyl group are downfield of the 3' whereas in 5'-MMT-G, the 3'-silyl ^1H and ^{13}C signals are the farther downfield [245]. This may be due to the greater steric crowding caused at the 2' position by a purine versus a pyrimidine ring or by the shielding ring current effects of the purine moiety. As no such trend is observed in the ^{29}Si chemical shifts, it seems probable that inductive effects, such as the ones postulated above, have a greater influence on the chemical shifts of the monosilylated derivatives. However, the chemical shifts in the 2',3'-disilylated compounds studied

appear upfield of their positions in the monosilylated derivatives. The greater shielding (2-4 ppm) might be due to the steric crowding between the 2'- and 3'-TBDMS groups or to changes in the nucleoside sugar ring conformation.

Upon standing in methanol, the N-benzoyl group of 5'-TBDMS and 2'-TBDMS derivatives of cytidine is cleaved [245]. In the case of N-Bz-5'-MMT-2'-TBDMS-C , this occurs to 98% over a 24 hour period. While debenzoylation in methanol is apparent for other nucleosides, the extent is virtually negligible relative to that observed for cytidine derivatives. It has been postulated that a pocket may form between the 2'-silyl group and the pyridine ring of cytidine in which a molecule of methanol may be inserted [245]. As this involvement might be reflected in the ^{29}Si chemical shift of the 2' isomer in deuterated methanol, the spectra of the cytidine derivatives were recorded in both $CDCl_3$ and CD_3OD as were those of the uridine derivatives, for purposes of comparison (peak positions in CD_3OD are in parentheses). The difference observed in going from $CDCl_3$ to CD_3OD is a shielding of the silicon signal by 1.3 to 1.8 ppm. However, the N-Bz-5'-MMT-2'-TBDMS-C signal is shielded by only 0.4 ppm. The smaller upfield shift for the 2'-silyl isomer of the protected cytidine implies the existence of an added deshielding effect in this molecule or the absence of a shielding effect in the other derivatives. However, these effects are relatively small and while they suggest a different solvent interaction with the 2'-silyl group of the cytidine derivative they do not prove this is the case. Furthermore, it is interesting to note the negligible effect of a change in solvent on the chemical shifts of the disilylated derivatives. It is possible that solvent effects are minimal in crowded molecules such as these.

3.2.3 Characterisation of Silylated Nucleosides

The chemical shifts presented in Table XIX do not constitute an exhaustive listing of peak positions for all possible TBDMS and TiPS nucleoside derivatives. They do, however, illustrate the range of shifts for both groups and give an idea of relative effects for different patterns of substitution.

In monosilylated ribonucleosides, the 2' signal is consistently downfield of the 3' and 5' peak positions. The 3'-silyl peak is also downfield of the 5' although this difference is negligible in many cases. On the basis of remote substituent effects, the 3' peak position is expected to be downfield of the 5' as there are two δ electronegative substituents in the former case and only one in the latter. The small difference between the 3'- and 5'-silyl peak positions may be due to the greater steric crowding felt at the 3' position. In the thymidine monosilylated derivatives, the difference between the 3' and 5' shifts is considerable (5.8 ppm). The 3'-silyl peak position is upfield and the 5'-silyl slightly downfield of the equivalent signals in ribonucleosides. This may reflect the absence of the 2'-hydroxyl deshielding effect on the 3'-silyl group and possibly less crowding at the 5' position.

The spectra of the monosilylated arabinonucleosides (A and C) were recorded in CD_3OD as these compounds are relatively insoluble in chloroform. The peak positions of the equivalent ribonucleosides are also reported in CD_3OD (values in parentheses). Although the chemical shifts of 2'- and 5'-silyl groups are very near to those reported for ribonucleosides, the 3'-silyl peak positions are shifted upfield for both arabinonucleosides studied. The reasons for these shift differences are not immediately apparent as in both the ribo- and the arabinonucleosides, the 3'-

TABLE XIX

^{29}Si Chemical Shifts of Silylated Nucleoside Derivatives

Nucleoside	5'TBDMS	3'TBDMS	2'TBDMS	3'5'TBDMS	2'3'TBDMS	2'3'5'TBDMS
T	26.4	23.6		22.0 20.1		
U	25.7	25.9	26.5			22.9 21.5 21.0
C	23.8 (22.0)	24.5 (22.8)	26.0 (23.9)			
N-Bz-C	22.7	23.84	25.7		22.1 21.2	
A	24.6 (22.4)	24.8 (22.7)	25.7 (23.6)		22.6 20.6	
N-Bz-A	23.9	24.6	25.8			
G	21.9	23.7	26.2			21.9 20.4 20.1
N-Bz-G	22.2	23.6	26.3		22.6 21.0	22.7 21.8 21.0
araA	(21.5)	(20.1)	(23.3)			
araC	(22.0)	(21.5)	(23.2)			

δ reported in ppm relative to TMS. Spectra were recorded in $CDCl_3$; values in parentheses were obtained in CD_3OD.

silyl group is exposed to a electronegative effect due to the presence of a 2'-hydroxyl group. The upfield shift may result from the different orientation of the 2' group or from a conformational change in the furanose ring. Both the ribose and the arabinose rings exist in a conformational equilibrium between the N and S forms (Figure 11) and it is known that a 2' or 3' substituent introduces geometrical constraints which may

stabilise one form over the other [249]. The relative population varies with the nature of the substituent and is also solvent and temperature dependent. N/S ratios can be estimated from the $J_{H1',H2'}$ and $J_{H3',H4'}$ values [248, 252]. Thus, a partial explanation of 3'-silyl shifts might be obtained from the determination by ^1H NMR of the predominant conformer in methanol-d4 of each compound studied.

The correct assignments of the di-TBDMS ribonucleosides were achieved with the ^1H-^{29}Si HETCOR technique. This method has been applied to the characterisation of a trimethylsilylated β-D-glucopyranoside by Nardin and Vincendon [275]. The experiment allows the correlation of a silicon signal to that of the protons to which it is coupled. By selection of appropriate parameters, the silicon signal can be linked to the corresponding sugar ring proton(s) (2', 3', 5'). As in the INEPT experiment, the intensity of the signal observed by the HETCOR technique is determined by the number of coupled protons as well as the coupling constant ($J_{Si,H}$) selected for the calculation of delay times. The optimisation of the experimental parameters is described in the appendix (section IV). As can be seen from the ^{29}Si-^1H HETCOR spectrum of 3',5'-diTBDMS-U reproduced in Figure 15, the parameters used for correlation of the silicon signals (reproduced vertically) with sugar ring protons also allow correlation with the more numerous silyl group t-butyl and dimethyl protons. Like the INEPT experiment, the HETCOR pulse sequence comprises a transfer of polarisation to the observed nucleus from the protons to which it is coupled. Signal enhancement increases with the number of coupled protons. Thus, despite optimisation of the experimental parameters for the ribose protons, the correlation signals of the more numerous silyl group protons are the most intense.

Figure 15

^{29}Si-^{1}H HETCOR Spectrum of 3',5'-Di-t-Butyldimethylsilyluridine

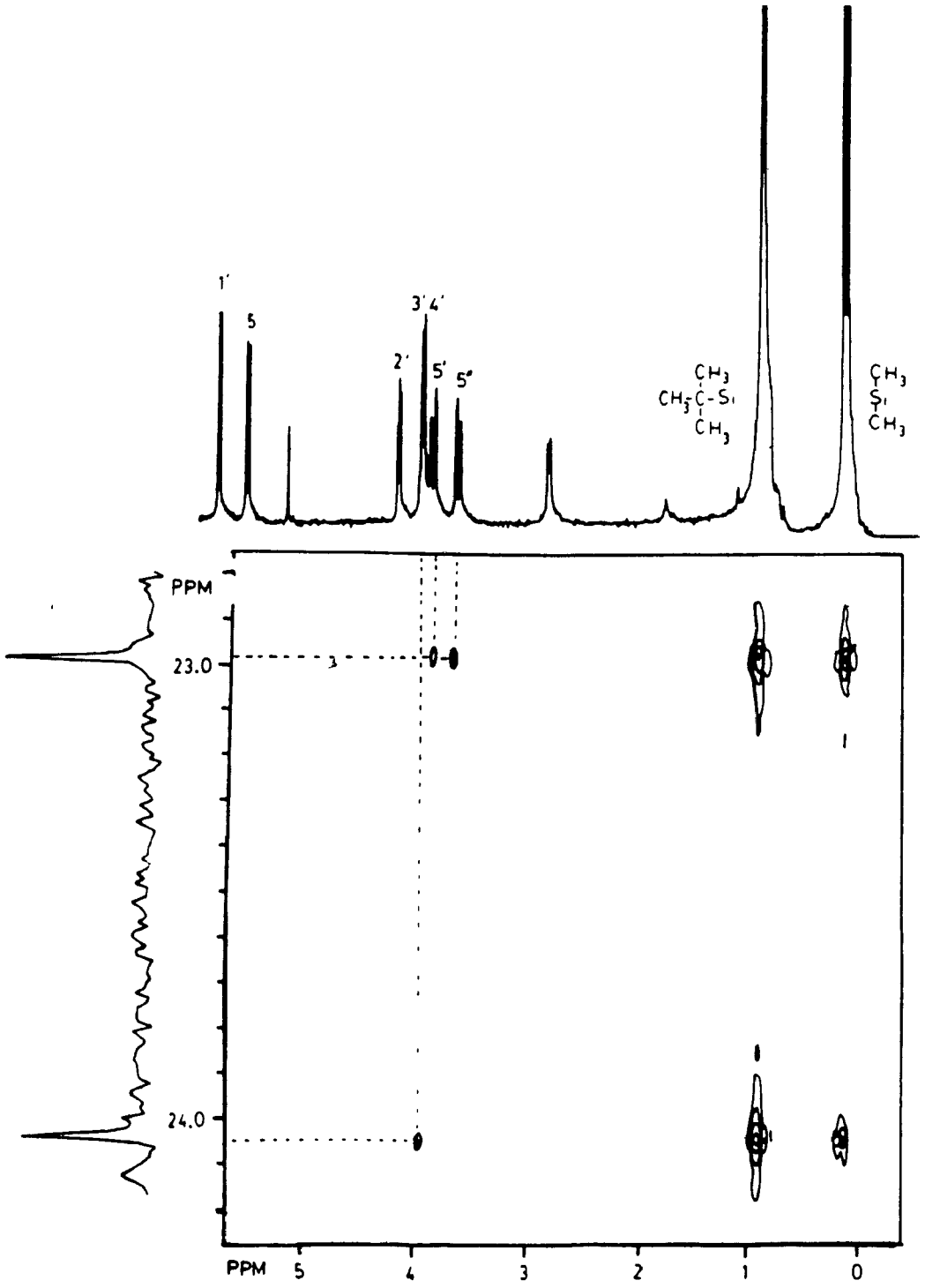


TABLE XX

^{29}Si Chemical Shifts of 2'5' and 3'5' Di-t-butyldimethylsilylated Nucleosides

Nucleoside	2'	5'	3'
2',5'TBDMS-U	25.4	23.2	
3',5'TBDMS-U		23.0	24.0
2',5'TBDMS-C	24.6	22.4	
3',5'TBDMS-C		22.3	23.2
N-Bz-2',5'TBDMS-C	26.5	23.9	
	(25.2)*	(22.1)*	
N-Bz-3',5'TBDMS-C		23.3	22.7
		(21.7)*	(21.1)*
2',5'TBDMS-A	26.2	23.4	
	(23.1)	(22.3)	
3',5'TBDMS-A		23.6	22.8
		(22.7)*	(22.1)*
N-Bz-2',5'TBDMS-A	25.4	22.7	
N-Bz-3',5'TBDMS-A		24.5	22.3
2',5'TBDMS-G	24.6	22.4	
3',5'TBDMS-G		23.2	22.3
N-Bz-2',5'TBDMS-G	25.2	22.9	
N-Bz-3',5'TBDMS-G		24.7	22.8
2',5'TBDMSaraA	23.2*	21.1*	
	(23.2)*	(21.5)*	
3',5'TBDMSaraA		21.0*	23.1*
		(20.8)*	(23.0)*

δ were reported in ppm relative to TMS. Spectra were recorded in CDCl3. Values in parentheses were recorded in CD3OD.
* These peak positions may be inverted.

From the data presented in Table XX, it is apparent the 2'-silyl signal is consistently upfield of the 5' in these derivatives whereas the relative positions of the 3' and 5' signals vary depending on the nucleoside. The 3'-silyl peak positions are shifted upfield (0.9 to 2.9 ppm) relative to the values observed for the monosilylated compounds. Conversely, no general trend can be discerned in the chemical shifts of the 5' and 2' groups in the disilylated derivatives relative to their positions for the monosilylated compounds.

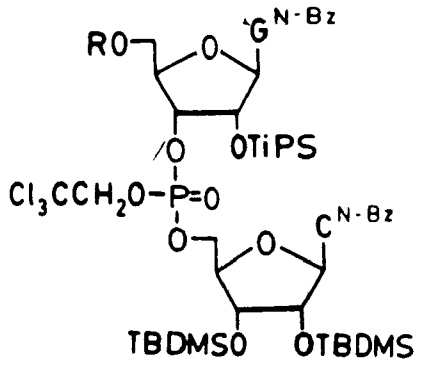
The ^{29}Si INEPT technique was also applied to the characterisation of a GpC dinucleotide (**109, 100**) containing one TiPS and two TBDMS groups (Table XXI). Unfortunately, it was not possible to observe all silicon signals in a single experiment as the TiPS and TBDMS silicon nuclei require different parameters for INEPT enhancement. Due to the chirality of the phosphotriester, dinucleotides exist as two diastereomers. Thus, two signals appear for the guanosine residue TiPS group. Only three peaks are observed for the two silyl groups of cytidine in the spectrum of each dinucleotide. However, one peak (22.43 ppm) is roughly double the intensity of the other TBDMS signals. The dinucleotide chemical shifts differ from their respective values in the corresponding mononucleosides (Tables XVIII and XIX). The guanosine TiPS signals are upfield of their position in the free nucleoside (0.6 to 0.9 ppm) possibly due to the increased steric crowding. Conversely two of the cytidine signals appear slightly downfield of the peak positions reported for N-Bz-2',3'-diTBDMS-C.

TABLE XXI

δ ^{29}Si of MMT-GpC and GpC [a]

compound	TBDMS	TiPS
109	22.51	17.72
	22.46	17.50
	22.43	
110	22.52	17.51
	22.48	17.36
	22.43	

a) Spectra recorded in $CDCl_3$ and δ reported in ppm relative to TMS. Digital resolution: 0.03 ppm.



109 **110**

R = MMT H

3.3 Conclusion

^{29}Si INEPT NMR has proved to be a rapid and efficient technique for the characterisation of silylated nucleosides. The identification of ribonucleoside 2'- and 3'-silyl isomers by ^{1}H or ^{13}C NMR requires multiple decoupling experiments or the use of the time consuming ^{1}H-^{13}C HETCOR technique. With ^{29}Si INEPT NMR, it is possible to easily differentiate such isomers in a matter of minutes on the basis of chemical shifts as 2'-silyl peak positions are consistently downfield of the 3' and 5' signals. This observation notwithstanding, few general conclusions can be drawn regarding trends in ^{29}Si chemical shifts. Peak positions remain difficult to predict or justify as the determining factors and their varying degree of importance are not completely understood.

However ^{29}Si NMR may have potential for use in the characterisation of protected ribonucleotides. The higher field signals observed for the TiPS group render guanosine residues easily distinguishable from other nucleosides bearing TBDMS groups. Furthermore, the wide shift dispersion of ^{29}Si chemical shifts might be exploited by protecting the 2'-hydroxyl of each nucleoside with a different silyl group. One drawback encountered in the study of dinucleotides **109** and **110** is the large resolution limit relative to the frequency differences of the 3' terminal residue TBDMS signals. This problem could easily be overcome by slightly modifying experimental parameters. A longer acquisition time (AT = 2.5 vs 0.8 s) would allow greater digital resolution (DG = 0.01 vs 0.03 ppm). However this would also lengthen the INEPT experiment if an equivalent signal to noise ratio is to be attained. The resolution would also be improved by performing experiments on a higher field instrument.

CHAPTER FOUR

Investigation of2,4-Dinitrobenzenesulfenyl as a 5'-Hydroxyl Protecting Group for Use with the Phosphodichloridite Procedure

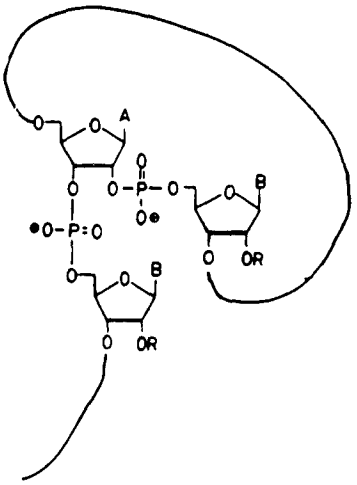
4.0 Introduction

Protecting groups to be used for the blocking of hydroxyl functions during ribonucleoside synthesis should be compatible with acid labile (MMT, DMT, THP, MTHP) as well as base labile (RCO) 3'- and 5'-hydroxyl protecting groups. The combination of the 5'-trityl and 2'-TBDMS groups with the phosphodichloridite coupling procedure has proved effective [123]. The susceptibility of N-acylated purines to glycosidic bond cleavage under the acidic conditions needed to remove a 5'-trityl group is less severe in the ribonucleotide area than in deoxyribonucleotides. However, as the synthesis of nucleotides becomes more refined, longer sequences are being produced. The problem of glycosidic bond cleavage becomes more important with the great number of repeated detritylations required for the synthesis of a long ribonucleotide. Mild acidic conditions which minimise depurination are commonly used for detritylation. Nevertheless, a 5'-hydroxyl protecting group which could be removed under neutral conditions would provide a useful alternative. Moreover, the use of such a group would facilitate the synthesis of molecules similar to the newly discovered lariat mRNA [276]. These structures, intermediates in the processing of mRNA, contain a branched trinucleotide consisting of a nucleoside (A) possessing two vicinal 2',5' and 3',5' phosphodiester linkages (Figure 16). In addition to the ribonucleotide loop linking the 2' and 5' posi-

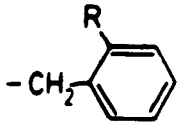
tions, a ribonucleotide tail extends in the 3' 5' direction. The synthesis of such a branched trimer in which both 3' terminal nucleosides bear identical 2'- and 3'-hydroxyl protecting groups has been reported [277, 278]. However, this molecule must be selectively extended from the 5' extremity as well as from both 3' positions. Selective nucleotide chain extension of the trimer may require as many as three different hydroxyl protecting groups in addition to the one needed for permanent blocking of the 2' positions.

Figure 16

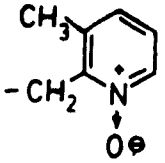
Branched Trinucleotide Structure of Lariat RNA



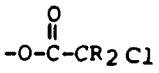
In addition to the previously described TBDMS function, a few groups which can be removed under neutral conditions have been investigated for hydroxyl protection, notably the benzyl (**111**), o-nitrobenzyl (**112**), 1-oxido-2-picolyl (**113**) and chloroacetyl (**114, 115**) groups [279]. The cleavage of these functions is often difficult and accompanied by undesired modifications of nucleoside bases or sugar moieties. Even silyl groups which are efficient for permanent 2'-hydroxyl protection, are not suitable for 5'-protection. Phosphotriesters are slightly sensitive to the fluoride ions



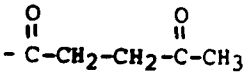
	R
111	H
112	NO_2



113



	R
114	H
115	Ph



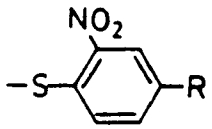
116

used to cleave silyl ethers [280] hence the phosphorous protecting group is usually removed prior to the silyl removal. The levulinyl group (Lv) (**116**) [281] is easily introduced by treatment with DCC and levulinic acid [115] and is rapidly cleaved with hydrazine hydrate in pyridine/acetic acid [115, 282]. The Lv group is compatible with N-acyl, DMT and TBDMS groups as the conditions required for its removal are essentially neutral and do not lead to deacylation, detritylation or silyl group isomerisation between the 2' and 3' positions [282, 283]. The group has been used for both 5'- and 3'-hydroxyl protection and is stable to the conditions required for the phosphodichloridite nucleoside coupling procedure [115, 238, 284]. The Lv group presents one drawback. It does not significantly affect the chromatographic properties of nucleosides and consequently, its introduction or removal is not easily monitored.

The 2,4-dinitrobenzesulfenyl group (DNBS) (**117**), introduced by Kharasch *et al.*[285] was first used for nucleoside hydroxyl protection in the preparation of 3'- and 5'-DNBS-T by Letsinger and co-workers [286]. The group is easily cleaved at room temperature by nucleophilic agents such as

sodium thiosulfate or thiophenol in neutral aqueous/alcoholic solutions or in pyridine [286]. Dinitrobenzenesulfenic esters were found to be stable to acetic acid as well as pyridine solutions of p-toluenesulfonyl chloride (TPS) or DCC, condensation reagents used in the phosphate diester and triester coupling procedures [286]. The closely related 2-nitrobenzenesulfenyl group (NBS) (**118**) has been applied to the protection of amino acid α-amine groups as well as the exocyclic amine functions of cytosine, adenine and guanine in both ribo- and deoxyribonucleosides [287]. The NBS group was

	R
117	NO_2
118	H

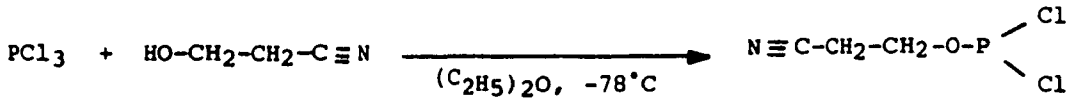


found to reduce the susceptibility of deoxynucleosides to depurination under acidic conditions; the half life of dA in 80% acetic acid at room temperature was increased from 30 minutes to 24 hours when the N-benzoyl protecting group was replaced by N-NBS [287]. In addition, N-NBS nucleosides were found to be stable to TBAF, hydrazine hydrate and 5% toluenesulfonic acid; the group is therefore compatible with silyl, levulinyl as well as trityl protecting groups [287]. As nitrobenzenesulfenyl esters might possess these properties in addition to their facile removal under neutral conditions, the feasibility of using the 2,4-dinitrobenzenesulfenyl group for the 5'-hydroxyl protection of ribonucleosides was investigated. The compatibility of the group with the phosphodichloridite coupling procedure was also evaluated.

4.1 Stability of the 2,4-Dinitrobenzenesulfenyl Group to the Phosphodichloridite Procedure Coupling Conditions

The first step undertaken was the preparation of a 5'-DNBS protected nucleoside to be submitted to the conditions of phosphodichloridite coupling as well as those required for oxidation of the phosphite triester produced to a phosphate triester. Uridine was selected for the study as this nucleoside does not possess an exocyclic amine group and thus is more easily protected for nucleotide synthesis. The cyanoethylphosphodichloridite was used in these experiments since the cyanoethyl group is easily cleaved at the end of nucleotide synthesis and this step is not accompanied by side reactions. The dichloridite (119) ($CEOPCl_2$) was prepared in 48% yield according to reported procedures [134, 140], by treatment of phosphorus trichloride with 3-hydroxypropionitrile in dry diethyl ether at -78°C.

Scheme XXXI



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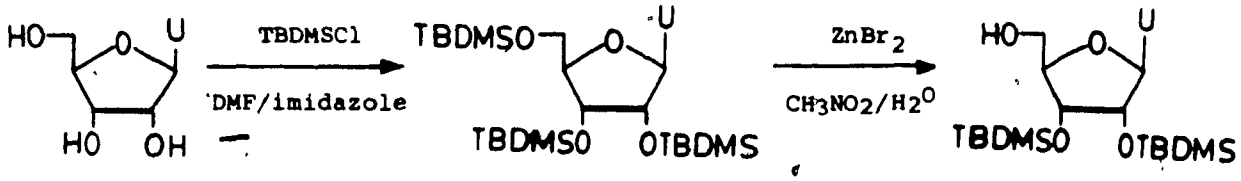
The DNBS group is usually introduced by reaction of the nucleoside with 2,4-dinitrobenzenesulfenyl chloride (DNBSCl) in pyridine. However, selective substitution in the 5' position is not easily achieved. In their early work with deoxyribonucleosides, Letsinger and co-workers found that while an acceptable yield (45%) of 5'-DNBS-dA was obtained when performing the reaction at 20°C, no such selectivity was achieved with Thymidine (T) [288]. It proved necessary to repeat the procedure at 0°C to obtain selectivity for reaction in the 5' position of T. Although a mixture of 3'-

DNBS, 3',5'-diDNBS and 5'-DNBS-T was still produced, the latter compound was predominant and was isolated in 37% yield [288]. The lack of selectivity observed for sulfenylation was expected to be a greater problem in ribonucleoside protection than in that of deoxyribonucleosides due to the presence of an additional reactive site, the 2'-hydroxyl group. In order to avoid multiple sulfenylation and the difficulties associated with separation of the products, the reaction was performed on 2',3' protected uridine (Scheme XXXII). The TBDMS group was selected for this purpose as

Scheme XXXII



120 121 122

silylated nucleoside derivatives are easily prepared and purified. The 2',3',5'-triTBDMS uridine (121) derivative was first prepared as described by Ogilvie *et al.*[123] by reaction of uridine with t-butyldimethylsilyl chloride in the presence of imidazole using anhydrous DMF as solvent. The product was isolated in 85% yield as a white solid. ^1H NMR peak integrations confirmed the presence of three TBDMS groups. The physical properties and ^1H NMR characteristics of the compounds prepared are summarised in Tables XXII and XXIII. It is interesting to note an additional splitting of H-5 for compound 121 and several of the other uridine derivatives due to coupling with the base NH group. The 5'-TBDMS group was selectively cleaved according to a procedure described by Seela *et al.*[141] by treatment of 121 with zinc bromide ($ZnBr_2$) (6 eq.) in an aqueous nitromethane solu-

TABLE XXII

Physical Properties of Derivatives 121-124

Product	#	Yield (%)	R_f	Melting Point (°C)	λ_{max} (EtOH) (nm)	λ_{min} (EtOH) (nm)
2'3'5'Si	121	85	0.53 a	105-106	262	232
2'3'Si	122	87	0.39 a	223-225[1]	263	232
5'DNBS-2'3'Si	123	51	0.72 b	213-215	325 264	301 232
N-DNBS-2'3'5'Si	124	22	0.75 c	205 d	308 264	298 233

1) Literature melting point: 224-226°C (ref. 123). TLC solvents: a) Et2O/ CH2Cl2 (1:2); b) EtOAc/Hexanes (1:2); c) Et2O/ Hexanes (2.1).

TABLE XXIII

^{1}H Chemical Shifts and Coupling Constants (J) of Derivatives 121-124

	H-6	H-5	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''
121	8.01 d (8.2)	5.65 dd (8.1) (2.3)	5.84 d (3.2)	4.04 m	4.04 m	4.04 m	3.96 d (11.8)	3.72 d (11.7)
122	7.60 d (8.0)	5.72 d (8.0)	5.44 d (5.5)	4.55 dd (5.2) (1.1)	4.16 dd (3.9) (1.1)	4.07 m	3.93 d (11.8)	3.73 d (9.8)
123	7.57 d (8.2)	5.70 dd (8.1) (2.2)	5.66 d (3.2)	4.36 m	4.20 m	4.20 m	4.20 m	
124	8.24 d (8.3)	5.89 d (7.9)	5.90 d (2.0)	4.09 m	4.09 m	4.09 m	4.02 d (12.5)	3.77 d (11.7)

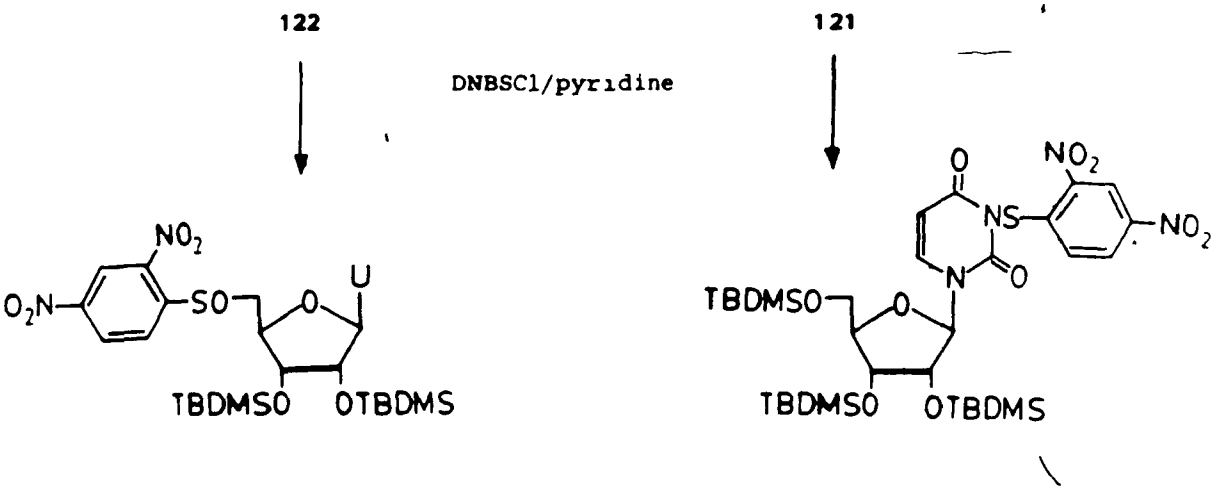
Spectra recorded in CDCl3. δ reported in ppm relative to TMS. Coupling constants (J) reported in Hz.

tion. Desilylation proceeded very slowly at room temperature and it became necessary to heat the reaction to 60°C as well as add more $ZnBr_2$ (2 eq.). Although starting material was still apparent by TLC, the reaction was stopped after 7 hours because of the appearance of a third compound re-

sulting from removal of two silyl groups. The desired 2',3'-diTBDMS-U (**122**) was isolated as a white solid in 87% yield. The presence of two silyl groups was confirmed by ^{1}H NMR relative peak integrations and the physical properties were identical to those reported in the literature 123.

The sulfenylation of compound **122** was performed in pyridine at room temperature. However, despite the presence of a single reactive hydroxyl group, three products were formed. The sulfenylated derivatives are not easily visualised by TLC as they tend to leave a strongly yellow coloured trail which is also UV absorbing. This streaking is responsible for the difficulty of cleanly separating products by column chromatography. Nevertheless, the desired 5'-DNBS-2',3'-diTBDMS-U (**123**) was isolated in 51% yield as a yellow solid. ^{1}H NMR revealed a downfield shift (ca 0.9 ppm) of the H-5' and H-5" peaks relative to their positions for **122**. The signals characteristic of the sulfenyl group were also present (9.13 ppm, H-3; 8.51 ppm, H-5; 7.95 ppm, H-6). The two other sulfenylated derivatives could not be separated due to their similar chromatographic properties. Furthermore, the products could not be unequivocally identified when the mixture was studied by ^{1}H NMR. However, the peak integrations revealed the ratio of benzenesulfenyl groups to ribose rings to be greater than one and this suggests the presence of disulfenylated material. Base sulfenylation of uridine was confirmed as shown in Scheme XXXIII, by treatment of 2',3',5'-triTBDMS-U (**121**) with DNBSCl. The reaction was initially attempted at 0°C but the starting material failed to react after more than one hour. The mixture was then allowed to warm to room temperature and was stirred for three more hours. A single product was formed in small quantity after this time and was isolated as a yellow powder. The product was

Scheme XXXIII



123 (51%) **124** (22%)

tentatively identified as N-DNBS-2',3',5'-triTBDMS-U (**124**) by ^1H NMR which revealed the absence of the base NH-3 signal normally seen around 9 ppm. The UV absorption maxima for this compound (λ_{max} = 304, 264 nm) differ from those reported for compound **123** (λ_{max} = 325, 264 nm). However despite direct substitution of the base, the characteristic pyrimidine absorption maximum remains unaffected whereas a shift is apparent for the sulfenyl absorption value. While this work was in progress the reaction of DNBSCl and a number of other sulfenyl chlorides with 2',3',5'-triacylated uridine was reported by Chattopadhyaya et al.[289]. Products were identified as N-3 derivatives by ^{13}C NMR which revealed minor base carbon chemical shift differences relative to unsubstituted uridine [289]. In light of these developments and the known lack of selectivity of DNBSCl reactions, it is probable the two side products isolated when treating **122** with the chloride were N-DNBS-2',3'-diTBDMS- and N,5'-diDNBS-2',3'-di-TBDMS-U. However, formation of these compounds should be minimised by performing reactions at low temperature as it has been shown, in the

reaction with **121**, that N-3 substitution does not occur at 0°C.

Like the exocyclic amine DNBS derivatives of A, G and C [287], N-DNBS-U is stable to acid solutions (5% TSA/H_2O and 80% CH_3COOH) but contrary to these compounds, N-3 substituted uridine is not stable to TBAF in dry pyridine at room temperature (1 minute half life) [289]. Thus, as the presence of side products severely complicates the purification of desired sulfenylated derivatives, it might be advantageous to select functions other than silyl groups for 2'- and 3'-hydroxyl protection and to treat crude reaction mixtures with TBAF prior to purification.

5'-DNBS-2',3'-TBDMS-U (**123**) was then submitted to the conditions required for nucleoside condensation and phosphite triester oxidation. A solution of compound **123** in anhydrous THF was treated with $CEOPCl_2$ (**119**) in the presence of 2,4,6-trimethylpyridine. After 20 minutes at 0°C and 90 minutes at room temperature followed by usual reaction work-up, 86% of the starting material was recovered. A solution of **123** and collidine in THF was then treated with an iodine solution in THF/H_2O for 5 minutes. At this time, an aqueous sodium bisulfite solution was added dropwise and, after extraction into dichloromethane, the starting material was completely recovered. This confirmed the stability of the DNBS group to the conditions involved in nucleotide synthesis using the phosphodichloridite procedure.

4.2 Preparation of a 5'-Dinitrobenzenesulfenylated Nucleoside Suitably Protected for Coupling by the Phosphodichloridite Method

Having thus established the compatibility of the nitrobenzenesulfenyl ester with the phosphodichloridite procedure, the preparation of a 2' protected dinucleotide bearing a 5'-DNBS group was undertaken. The dimethoxytrityl group (DMT), which has been found compatible with the dichloridite procedure when used for 2'-hydroxyl protection [291], was selected for this study. While evaluating the DMT group for 2' protection, Décout and Ogilvie found that protection of the 3'-hydroxyl group of the 3' terminal nucleoside was not necessary as no 3',3' linked dimer was observed [291]. The exclusive reaction at the 5'-hydroxyl can be attributed to the much greater accessibility of this position relative to the 3'-hydroxyl which is hindered by the bulk of the 2'-DMT group.

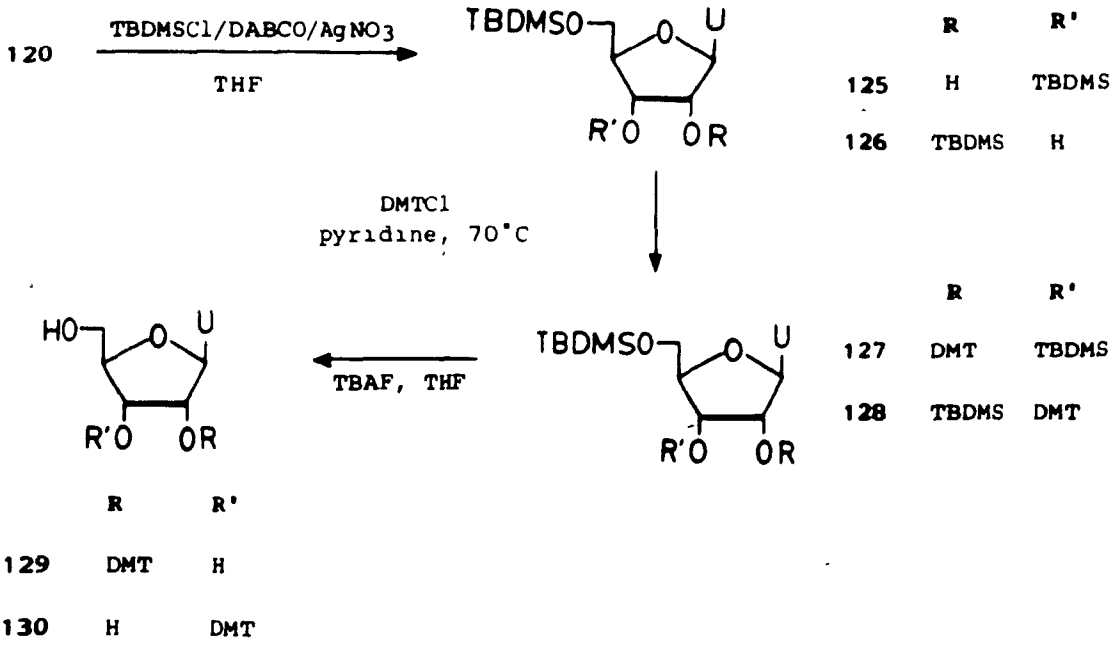
4.2.1 Preparation of 2'-Dimethoxytrityluridine (129)

In order to tritylate the 2'-hydroxyl of uridine, the 3' and 5' positions were first protected with TBDMS groups. The selective 3',5' silylation was attempted according to the procedure described by Ogilvie et al.[236]. Uridine (120) was treated with t-butyldimethylsilyl chloride in the presence of 1,4-diazabicyclo[2.2.2]-octane (DABCO) and silver nitrate (Scheme XXXIV). The best ratio of 3',5'-isomer to 2',5'-isomer obtained by this method (1:1) was not as high as that reported (10:3) and could not be improved despite repeated attempts. Because of the similar chromatographic properties of the two disilylated isomers, the separation of the near 1:1 mixture posed special difficulties. The isolation of the desired 3',5'-diTBDMS-U (125) from the faster moving 2',5'-isomer (126) required re-

peated purification by column chromatography. The best yield of pure **125**, isolated as a white solid, was 41%. The physical properties and ^{1}H NMR characteristics of this and the following compounds are summarised in Tables XXIV and XXV.

The product (**125**) was then tritylated by treatment with DMTCl in pyridine. As the 2' position is not easily accessible in the 3',5'-diTBDMS derivative for reaction with the bulky trityl group, it was necessary to use 3 equivalents of chloride and heat the reaction to 70°C for over 12 hours to form **126**. Unfortunately, under these conditions, isomerisation of the 3'-silyl group to the 2'position began to occur. The presence of 2',5'-diTBDMS-U (**126**) became apparent by TLC. Thus, despite the continued presence of starting material, the reaction was stopped to avoid tritylation of the 2',5'-isomer. The desired product (**127**) was isolated in 40% yield as a yellow solid after repeated purifications by column chromatog-

Scheme XXXIV

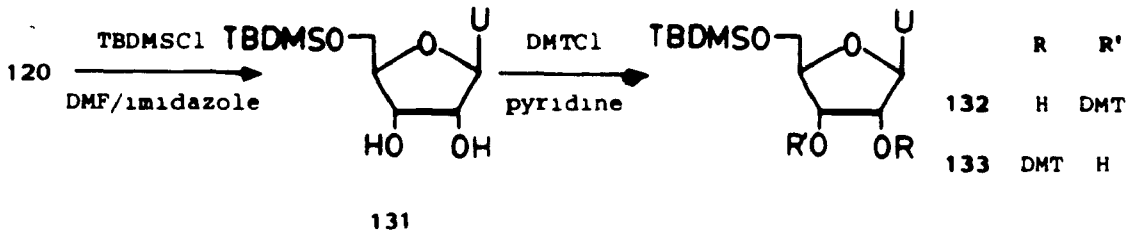


	R	R'
125	H	TBDMS
126	TBDMS	H
127	DMT	TBDMS
128	TBDMS	DMT
129	DMT	H
130	H	DMT

raphy to eliminate traces of 2',5'-diTBDMS-3'-DMT-U (128). In addition to possessing similar chromatographic properties which render their separation difficult, these isomers exhibit a marked tendency to detritylation during separation on silica gel. This was minimised by addition of traces of triethylamine to the eluting solvents. The two silyl groups of compound 127 were then removed by treatment with tetrabutylammonium fluoride (TBAF) [102, 103] and 2'-DMT-U (129) was obtained as a white solid in 74% yield. The structure of 129 was confirmed by ^{1}H NMR in deuterated DMSO. The spectrum revealed the presence of two free hydroxyl groups at 5.02 and 4.96 ppm and these were assigned to the 5' and 3' positions respectively by selective ^{1}H decoupling.

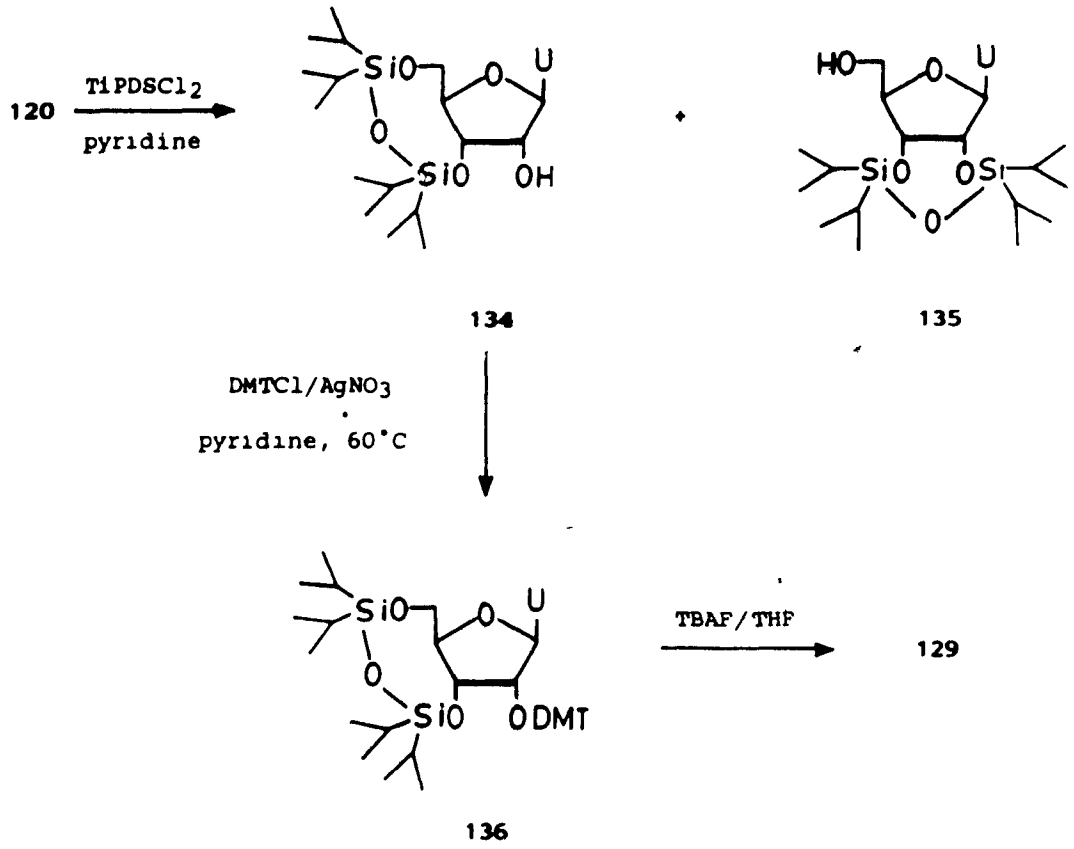
The procedure outlined above did not prove efficient for the synthesis of 2'-DMT-U as isomer separation generally required multiple purifications by column chromatography. Furthermore, the product was often contaminated with 3'-DMT-U (130) making it unsuitable for condensation reactions. In the hope that the disubstituted isomers 132 and 133 might be more easily separable by chromatography than derivatives 127 and 128, another approach was undertaken. Uridine (120) was first silylated in the 5' position. The product, a white solid, was obtained in 83% yield and was reacted with DMTCl in pyridine at room temperature. As the 2' position is less crowded in this derivative than in 125, tritylation occurred at room temperature. The desired product (132) and 5'-TBDMS-3'-DMT-U (133) were obtained as white solids in yields of 76% and 22% respectively. Unfortunately, the purification of these isomers was also difficult and traces of 133 were often visible in the ^{1}H NMR spectrum of the 2'-DMT derivative (132). The 5'-silyl group was removed as previously described, by treatment with TBAF, to afford 2'-DMT-U (129) in 88% yield.

Scheme XXXV



The bifunctional silylating agent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TiPDSCl_2$), described by Markiewicz *et al.*[291-293] selectively protects the 3' and 5' positions of ribonucleosides. The more reactive 5'-hydroxyl group is first blocked then the second silyl chloride reacts with the 3' position. In order to bypass the problems associated with isomer separation in the preparation of 2'-DMT-U, the approach outlined in Scheme XXXVI was undertaken. Uridine (**120**) was treated with $TiPDSCl_2$ in pyridine at room temperature under argon atmosphere to yield 70% of 3',5'-TiPDS-U (**134**) as an oil. The 2',3'-isomer was isolated in 8% yield. When characterising the products by ^1H NMR, it was observed that the H-1' signal, which is normally a doublet due to coupling with H-2', appears as a broadened singlet for both compounds. As previously discussed, the ribose ring conformation can be determined on the basis of $J_{1',2'}$ values. In these molecules, the coupling constant is negligible which implies the ring is locked in an N conformation (C-3'endo) by the disiloxane group. As in the case of 3',5'-diTBDMS-U (**125**), tritylation of the hindered 2'-hydroxyl group of **134** required heating and an excess of DMTCl. However, the unreacted chloride was hydrolysed at the end of reaction and the large quantities of tritanol thus produced hindered purification of the product (**136**). The problem was circumvented by using a variation of the silver nitrate/pyridine system described by Ogilvie *et al.*[236] for selective

Scheme XXXVI



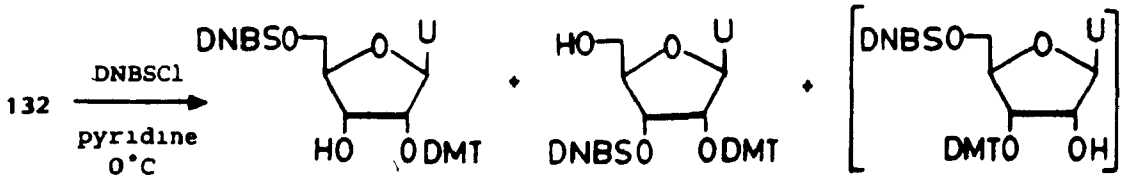
tritylation of ribonucleosides. Heating was also necessary with this procedure to effect reaction at the hindered 2' position. The product was obtained in 97% yield as an oil and was desilylated with TBAF as described earlier to afford pure 2'-DMT-U (129) in 98% yield.

4.2.2 Preparation of 5'-Dinitrobenzenesulfenyl-2'-Dimethoxytrityluridine

In order to minimise reaction of the sulfenyl chloride with the N-3 position of the base, 2'-DMT-U was treated with DNBSCl at 0°C. Side product formation was further reduced by dissolving the chloride in anhydrous pyridine and adding it dropwise to a solution of 2'-DMT-U. This ensured

predominant reaction at the 5' position which is less crowded than the 3'. Nevertheless, a mixture of disulfenylated compounds accounted for 8% of reaction yields and 3'-DNBS-2'-DMT-U (138) for 18%. The desired product (137) was isolated in 47% yield but purification was difficult as sulfenylated material was retained on silica gel and compounds did not separate cleanly. However, the chromatographic properties of the 3'-DMT derivative (139) are sufficiently different from those of 137 to allow their separation. Thus, it was possible to perform the reaction on starting material (132) containing traces of 3'-DMT-U (133). As observed previously, the proton signals are shifted downfield upon substitution with a DNBS group (Table XXV). The H-5' and H-5" signals for 5'-DNBS-2'-DMT-U (137) and the H-3' signals of 3'-DNBS-2'-DMT-U (138) are shifted downfield of their positions in 2'-DMT-U (129) by 0.4 and 0.82 ppm respectively. The characteristic shielding effect of a trityl group upon the H-2' proton is apparent in both 137 and 138 compared to the 3'-DMT derivative (139). The absence of H-5' and H-5" deshielding as well as H-3' shielding in 5'-DNBS-3'-DMT-U (139) compared to 3'-DMT-U (130) and the 2'-DMT derivative (137) respectively might be the result of steric crowding in these molecules. However, signals were observed in ^{1}H NMR for the free 3'-hydroxyl group of 138 (2.48 ppm) and the 2'-hydroxyl group of 139 (3.00 ppm). The H-6 signals of both 138 and 139 were obscured by the trityl peaks.

Scheme XXXVII



137 138 139

TABLE XXIV

Physical Properties of the Uridine Derivatives (Sections 4.2, 4.3)

Product	#	Yield (%)	R_f	Melting Point (°C)	λ_{max} (EtOH) (nm)	λ_{min} (EtOH) (nm)
3'5'Si	125	40	0.47 a	135-137[1]	264	232
2'5'Si	126	35	0.60 a	120-121[2]	264	232
3'5'Si-2'DMT	127	40	0.35 b	127-128	263 236	253 222
5'Si	131	83	0.22 c	135-138[3]	263	232
5'Si-2'DMT	132	76	0.53 d	118-119	264 234	252 223
5'Si-3'DMT	133	22	0.40 d	119-121	263 235	250 225
3'5'TiPDS	134	70	0.46 e	102-103	263	232
2'3'TiPDS	135	8	0.30 e	105-106	264	232
3'5'TiPDS-2'DMT	136	97	0.42 f	141-142	264 236	251 222
2'DMT	129	98	0.42 g	137-140	265 236	255 224
5'DNBS-2'DMT	137	47	0.45 e	115-117	327 263 235	295 255 225
3'DNBS-2'DMT	138	8	0.31 e	120-121	326 264 236	296 253 224
5'DNBS-3'DMT	139		0.31 e			
5'DNBS-UpU	141	50	0.38 h 0.30	157-159	325 264 237	295 254 225
3'3'UpU dimer	142	8	0.45 h	147-149	326 264 234	293 255 225
UpU	143	45	0.28 i	134-135	264 236	254 226

Literature Melting Points: 1) 136-137°C. 2) 121-122°C. 3) 136-139°C. (ref. 123). TLC solvents: a) Et2O/Hexanes (4:1); b)idem (2:1); c) EtOH/CH2Cl2 (0.5:9.5); d) Et2O/CH2Cl2 (2:1); e) idem (1:1); f) idem (1:3); g) EtOAc; h) CHCl3/EtOAc (1:9); i) EtOH/CH2Cl2/EtOAc (3:10:87).

TABLE XXV

^{1}H Chemical Shifts and Coupling Constants (J) of the Uridine Derivatives
(Sections 4.2, 4.3)

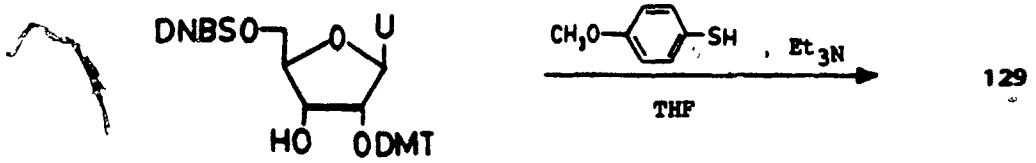
	H-6	H-5	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''
125	7.79 d (8.3)	5.68 d (8.0)	5.95 d (4.1)	4.02 m	4.25 dd (9.2)	4.02 m	3.93 d (11.7)	3.72 d (11.5)
126	8.02 d (8.2)	5.72 dd (8.0) (3.0)	6.00 d (4.1)	4.20 dd (4.5)	4.12 m	4.12 m	4.00 d (11.2)	3.82 d (11.2)
127	obs.	5.42 d (8.1)	6.21 d (7.3)	4.30 m	3.79 m	3.92 m	3.61 m	
128	obs.	5.68 d (8.1)	5.93 d (5.2)	obs.	obs.	obs.	obs.	
129	obs	5.68 d (7.8)	5.94 d (7.3)	4.60 dd (7.3) (4.9)	2.88 d (4.4)	4.02 m	3.67 d (8.3)	3.44 d (8.3)
130	obs.	5.66 d (8.8)	5.84 d (6.5)	4.38 m	obs.	obs.	obs.	
131	8.13 d (8.1)	5.70 d (8.2)	5.92 d (1.6)	4.26 m	4.26 m	4.19 m	4.06 d (11.7)	3.87 d (11.6)
132	8.06 d (8.2)	5.69 d (8.2)	5.88 bs	4.20 m	2.89 m	4.20 m	4.16 m	3.99
133	7.62 d (8.3)	5.60 dd (8.2) (2.7)	6.43 d (7.5)	4.23 m	3.21 m	4.13 m	4.00 m	3.85 m
134	7.76 d (8.5)	5.69 d (8.8)	5.74 s	4.26 m	4.17 m	4.17 m	4.14 d (12.3)	3.98 d (12.5)
135	8.05 d (8.3)	5.68 d (8.1)	5.67 bs	4.37 d (3.1)	4.23 m	4.18 bs	4.13 d (12.0)	3.95 d (11.8)
136	7.57 d (7.9)	5.43 dd (8.1) (1.3)	4.96 bs	4.52 dd (8.8) (4.5)	4.23 d (8.4)	4.55 m	4.14 dd (11.0) (1.1)	4.03 dd (13.4) (1.1)
137	7.09 d (8.1)	5.66 dd (8.0) (2.0)	6.09 d (7.0)	4.45 m	2.91 m	4.15 m	4.07 d (10.5)	3.89 dd (10.5) (3.5)
138	obs.	5.12 dd (8.0) (2.0)	6.61 d (7.9)	4.59 dd (8.0) (4.7)	3.70 dd (4.7)	4.05 m	3.78 m	
139	obs.	5.67 d (7.6)	6.05 d (8.0)	4.99 dd (8.2) (4.7)	3.21 d (4.5)	4.21 m	3.75 m	
141*	obs.	5.76 d (6.0) 5.60 d (7.8)	5.98 d (9.4) 5.84 d (8.0)	4.57 m	3.73 m	4.10 m	3.98 m	
142	obs. 6.84 m	5.50 d (6.7) 5.34 d (7.4)	6.09 d (7.0) 5.94 d (6.3)	4.80 m 4.63 m	2.68 m	4.63 m	4.30 m	
143*	obs.	5.41 m 5.35 m	5.98 m	4.72 m 4.59 m	3.11 m	4.06 m	3.89 m	3.56 m

Spectra recorded in CDCl3. δ reported in ppm relative to TMS. Coupling constants (J) reported in Hz. (*) Mixture of 2 diastereomers. Obscured signals indicated by obs.

4.2.3 Removal of the 2,4-Dinitrobenzenesulfenyl Group and Stability of the Cyanoethyl Protecting Group to the Reaction Conditions

Several means have been used for removal of the DNBS group from the hydroxyl functions of nucleosides. The nitrobenzenesulfenyl ethers have been cleaved by treatment with sodium thiosulfate in methanol/water or thiophenol in pyridine [286]. Treatment of a solution of nucleoside in pyridine with hydrogen sulfide has also proved effective [288]. The ideal conditions for removal of the DNBS group and the stability of the 2'-trityl group were determined by first treating 5'-DNBS-2'-DMT-U (137) with thiophenol in anhydrous pyridine at room temperature. The pyridine used as solvent made the reaction difficult to follow by TLC and rendered work-up and purification of a small scale preparation impractical. Furthermore, the procedure, although less lengthy than the hydrogen sulfide method, still required two to three hours for complete deprotection. Chattopadhyaya *et al.*[287] used triethylammonium thiocresolate in pyridine at room temperature under argon atmosphere for removal of a 5'-(2-nitrobenzenesulfenyl) group and reported complete cleavage after one hour. This method combines the advantages of shorter reaction times with the easier handling of reagents. The deprotection of compound 137 was repeated using this procedure substituting THF for pyridine in order to facilitate solubilisation of the starting material as well as TLC monitoring and reaction work-

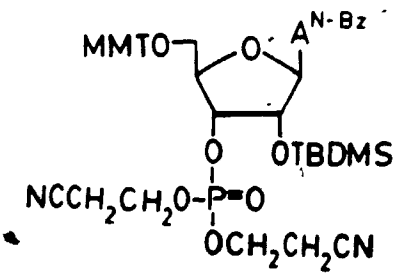
Scheme XXXVIII



137 129

up. After 20 minutes, sulfenyl removal was complete and 2'-DMT-U (129) was isolated in 60% yield following purification.

The cyanoethyl phosphorous protecting group is cleaved by treatment with triethylamine in acetonitrile at room temperature [131]. Removal of the DNBS group was attempted once again in the absence of triethylamine to determine if its use could be avoided. However, under these conditions, important loss of the 2'-trityl group from compound 137 was observed. This was estimated to be around 50% by TLC monitoring. The deprotection was then repeated in the presence of traces of pyridine to minimise detritylation but this rendered the reaction difficult to follow by TLC as the characteristic streaking of sulfenylated material was worsened considerably. Having established the necessity of adding triethylamine to the reaction mixture, the stability of the cyanoethyl group to the triethylammonium thiocresolate solution was evaluated. This was verified by treating the 3'-bis(cyanoethyl)phosphotriester of N-benzoyl-2'-TBDMS-A (140), prepared by M. Damha [132], with the reagent solution as in Scheme XXXVIII. Although the proportion of triethylamine is 8% in the 0.5M triethylammonium thiocresolate solution, this is diluted to approximately 2% in the actual reaction mixture. This concentration is far below that used for complete decyanoethylation which is 40%. The starting material (140)



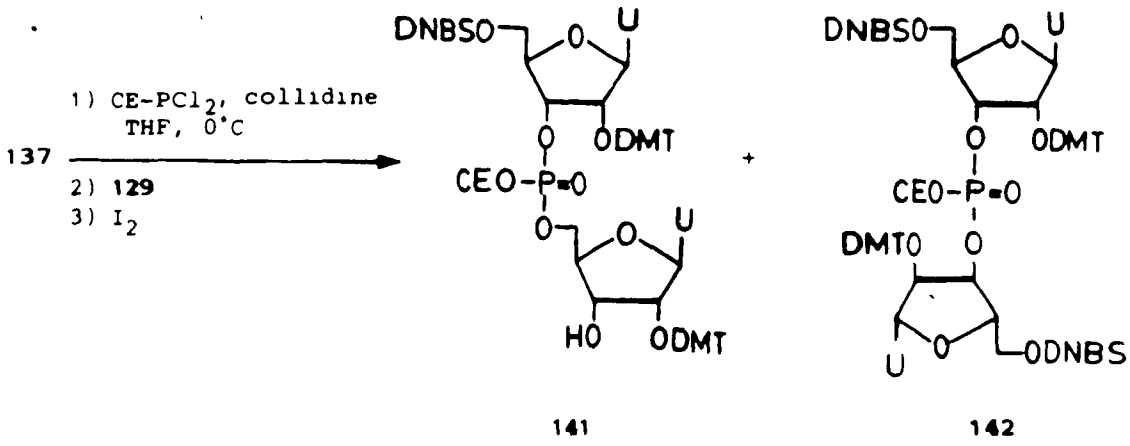
140

remained intact for over 30 minutes but after 45 minutes at room temperature, a slower moving tritylated compound was observed by TLC monitoring. This became the major component after 90 minutes and when the solution was allowed to stand overnight, no starting material remained. The appearance of slow moving tritylated material is consistent with decyanoethylation as the phosphate diester formed is much more polar than the triester. As removal of the DNBS group requires 20 minutes and as the cyanoethyl group is stable at the concentrations of reagents used for over 30 minutes, the use of these two functions remains a feasible combination for the synthesis of short nucleotide sequences.

4.3 Preparation and Characterisation of 5'-Dinitrobenzenesulfenyl-2'-DMT-Uridine-P($CH_2CH_2^{CN}$)-2'-DMT-Uridine (141)

The protected dimer (141) was prepared according to previously described procedures [123] (Scheme XXXIX). As the 3'-hydroxyl group is less reactive than the 5' group due to increased steric crowding, 5'-DNBS-2'-DMT-U (137) was first treated with the phosphodichloridite (119) in anhydrous THF at 0°C in the presence of collidine. The more reactive 2'-DMT-U was then added and, following oxidation of the phosphite to a phosphate by treatment with an iodine solution, the desired product was isolated in 50% yield as a yellow powder. The low yield is in part attributable to the difficulty encountered in the purification of the product by column chromatography on silica gel. It proved necessary to add traces of base to the eluting solvents to minimise detritylation. Pyridine was used in lieu of triethylamine to avoid any decyanoethylation but, as had been found for TLC separations, this base increased the difficulty in separating nitro-

Scheme XXXIX



141 142

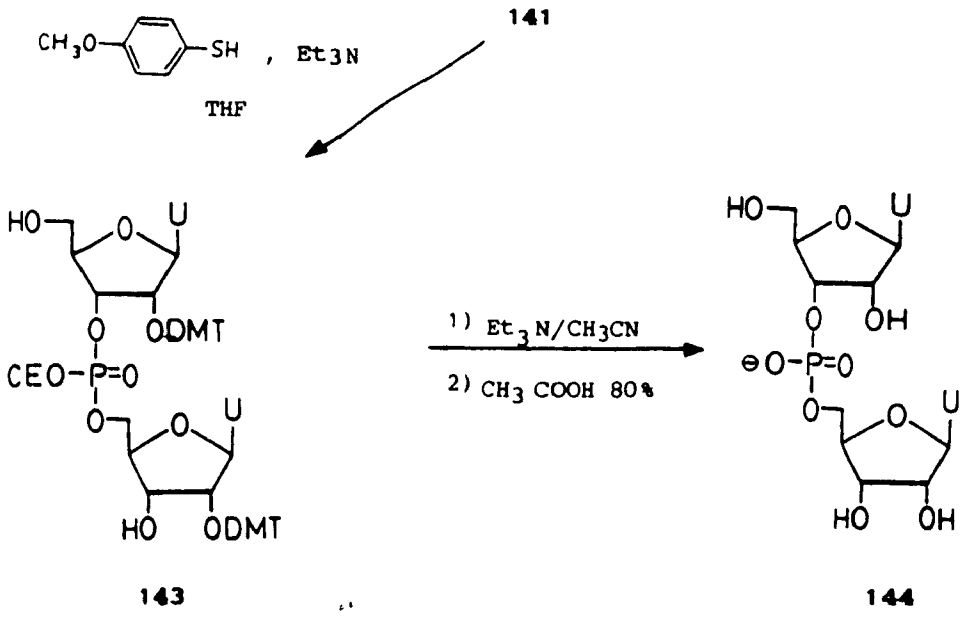
benzenesulfenylated compounds. Although no 3'-3' coupling had been reported in the absence of 3'-hydroxyl protection and when a 2'-DMT group was used [291], 3',3'-dimer (**142**) was obtained in 8% yield from this reaction. ^{1}H NMR characterisation of **141** revealed the presence of a single DNBS group whereas the integration of the corresponding signals showed two such groups for the 3',3'-dimer. The structure of this product (**142**) was further confirmed by ^{31}P NMR. As the two linked nucleosides are identical, there is no longer a chiral center at the phosphorous atom and a single peak is observed at -2.51 ppm. The non-equivalence of the H-5 and H-1' signals in this dimer are due to differences in their environment resulting from the conformation adopted by the molecule. The desired dinucleotide (**141**) exists as two diastereomers and two signals were observed by ^{31}P NMR at -2.37 and -2.71 ppm.

The product was then treated with triethylammonium thiocresolate by the previously described procedure to afford dinucleotide **143** in 45% yield. Once again the problems associated with the purification of these compounds contributed to lowering the quantities of recovered material. Removal of the DNBS group was verified by ^{1}H NMR. Following deprotection

of the 5'-hydroxyl group, the dinucleotide can be extended from this position.

In order to confirm the structure of **143**, the dinucleotide was fully deprotected. The β-cyanoethyl group was first cleaved from the triester by treatment with triethylamine in acetonitrile. At this point in the deprotection sequence, it becomes necessary to adopt sterile techniques for handling the product as the fully deprotected dinucleotide is sensitive to cleavage by the ubiquitous ribonucleic acid hydrolytic enzymes (RNases). However, removal of the 2'-trityl groups can be safely performed at this stage without risk of isomerisation or cleavage of the internucleotide bond. Phosphate diesters are more stable to acidic conditions than the triesters. The 2'-DMT groups were cleaved by treatment with acetic acid at room temperature for 1½ hours. The mixture was neutralised by addition of ammonium hydroxide and was evaporated to dryness at reduced pressure. Cellulose TLC analysis revealed the presence of a single product and the

Scheme XL



143 **144**

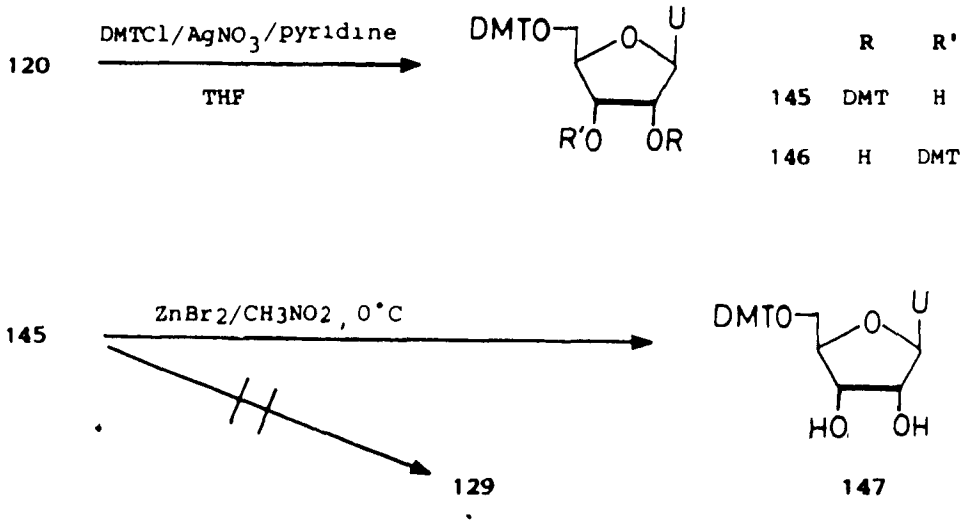
yield of fully deprotected dinucleotide (**144**) was estimated to be 54% by UV absorption (290 O.D. units). The product was purified by chromatography on cellulose plates to eliminate residual salts from the deprotection reactions and was recovered in 23% yield (122 O.D. units). The structure was then confirmed by enzyme degradation with spleen phosphodiesterase (Bovine, type II, EC 3.1.16.1). Uridine and 3'-uridine phosphate monoester were observed in a 1:1 ratio by HPLC analysis [294].

4.4 Reactions of Dimethoxytritylated Uridine with Zinc Bromide

In an effort to find a rapid and easy method of preparing 2'-DMT-U (**129**), an interesting interaction was observed between zinc bromide and tritylated ribonucleosides. The method outlined in Scheme XLI was attempted. The 2',5'-DMT derivative (**145**) was prepared according to a procedure developed in this laboratory for selective tritylation in the 2' position [236]. Uridine (**120**) was treated with DMTCl in THF in the presence of silver nitrate and pyridine. The 2',5'- and 3',5'-isomers, both white powders, were obtained in yields of 50% and 24% respectively. The compounds were easily distinguished by their ^{1}H NMR spectra in deuterated DMSO which revealed a 3'-hydroxyl signal at 4.77 ppm for **145** and a 2'-hydroxyl signal at 5.89 ppm for **146**. The physical properties and ^{1}H NMR characteristics of these and the following compounds are summarised in Tables XXVI and XXVII.

Under anhydrous conditions, zinc bromide is used for the selective removal of 5'-trityl protecting groups in deoxyribonucleosides leaving 3'-trityl groups intact [91]. It was thought its action would be the same on compound **145** thus yielding the desired 2'-DMT-U (**129**). However, this was

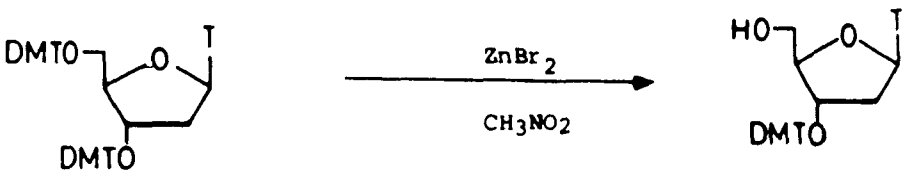
Scheme XLI



not the case, detritylation occurred exclusively in the 2' position. After 30 minutes at 0-5°C, no unreacted starting material remained and 5'-DMT-U (**147**) was isolated in 91% yield. The procedure was repeated on 5'-DMT-U (**147**) increasing the number of equivalents of $ZnBr_2$ from 5 to 12. Complete detritylation occurred after 10 minutes at 0-5°C affording a single product, uridine (**120**).

Reaction times of 10 minutes have been reported for complete and selective removal of a 5'-trityl group from a 3',5'-diDMT-deoxynucleoside at 0°C with a saturated solution of $ZnBr_2$ [348]. This result was confirmed by reaction of 3',5'-diDMT-T (**148**) (prepared by J. Cormier) with 10 equivalents of $ZnBr_2$ at 0°C. As reported, selective cleavage of the 5'-trityl group occurred and 3'-DMT-T was isolated in 90% yield. The structure was confirmed by ^1H NMR in deuterated DMSO which shows a signal due the 5'-hydroxyl group at 4.96 ppm. Furthermore, H-5' and H-5" are deshielded relative to the ditritylated derivative reflecting both the loss of the DMT group and the decrease of steric crowding.

Scheme XLII



148 149

TABLE XXVI

Physical Properties of the Uridine Derivatives (Section 4.4)

Product	#	Yield (%)	Rf	Melting Point (°C)	λmax (EtOH) (nm)	λmin (EtOH) (nm)
2'5'DMT	145	50	0.68 a	144-145[1]	266 233	260 223
3'5'DMT	146	24	0.47 a	136-137[2]	265 233	259 224
5'DMT	147	91	0.46 b	110-111	264 234	252 223
2'3'5'DMT	150	87	0.75 c	135-137	264 234	248 220
2'3'DMT	151	19	0.35 a	140-141	264 235	250 221

Literature Melting Points: 1) 144-146°C. 2) 136-138°C. (ref. 236). TLC solvents: a) Et2O/CH2Cl2 (1:1); b) EtOAc; c) Et2O/CH2Cl2 (1:3).

TABLE XXVII

^{1}H Chemical Shifts and Coupling Constants (J) of the Uridine Derivatives
(Section 4.4)

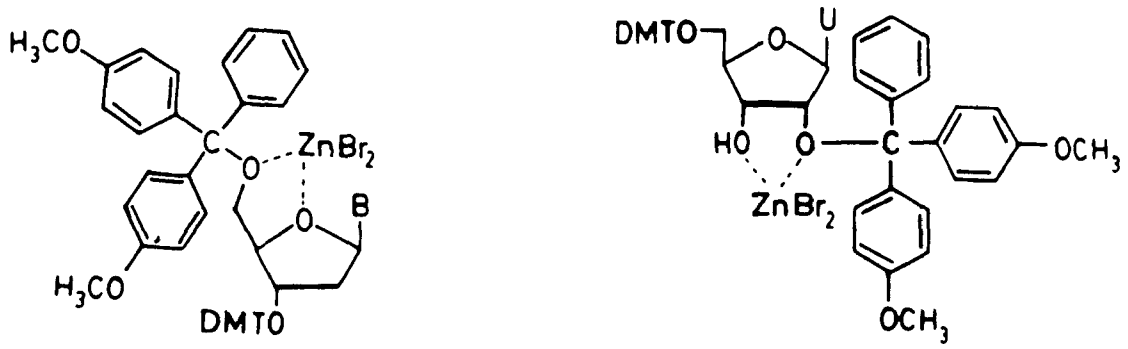
	H-6	H-5	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''
145	7.78 d (8.0)	5.18 dd (8.2) (1.6)	6.59 d (7.4)	4.51 dd (7.4) (4.4)	2.80 d (4.4)	4.01 bs	3.19 m	
146	7.62 d (8.3)	5.27 dd (7.9) (2.1)	6.01 d (5.2)	4.35 dd (4.8)	2.92 d (6.5)	3.86 m	3.35 dd (11.0) (2.1)	3.06 dd (10.8) (3.5)
129*	obs.	5.32 d (7.8)	6.08 d (7.2)	4.20 dd (7.3) (4.9)	3.13 dd	3.82 m	3.36 m	
147*	7.20 d (8.0)	5.30 d (8.0)	5.74 d (3.2)	4.07 m	4.07 m	3.94 m	3.23 m	
148*	obs.	---	6.14 t (2.0)	1.71 m 1.57 m	4.21 m	3.93 m	2.96 m	
149*	obs.	---	6.21 t (2.1)	1.70 m 1.52 m	4.26 m	3.78 m	3.36 m	3.31 m
150	7.59 d (8.3)	4.86 d (8.6)	obs.	4.59 dd (8.2) (3.4)	3.48 dd (4.4)	4.00 m	2.65 dd (10.4) (2.1)	2.39 d (10.9)
151	7.34 d (8.2)	5.50 d (7.9)	6.36 d (8.2)	4.78 dd (8.4) (4.2)	3.25 d (5.0)	3.84 m	3.15 d (12.2)	2.54 d (12.2)

Spectra recorded in $CDCl_3$ except (*) recorded in dmso-d6. δ reported in ppm relative to TMS. Coupling constants (J) reported in Hz.

The selective detritylation of deoxyribonucleotides has been attributed to the existence of a bidentate chelation site in these molecules [289]. The complexation of $ZnBr_2$ between the sugar ring oxygen atom and the 5' ether, represented in Figure 17a, would account for predominant reaction in the 5' position. However, the results previously presented indicate that the presence of an adjacent hydroxyl group to the 2'- or 3'-trityl group interferes with selective 5'-detritylation. The preference of

Figure 17

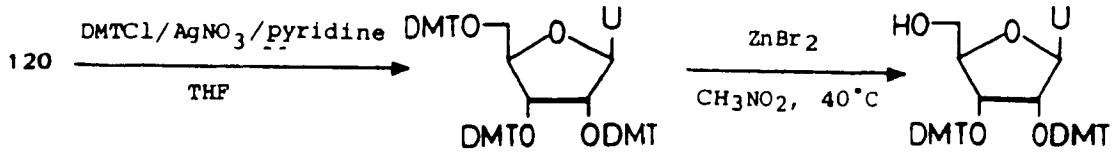
Possible Bidentate Chelation of Zinc Bromide with Nucleosides



$ZnBr_2$ for the alternative chelation site present in ribonucleosides (Figure 17b) would explain the selectivity of reaction in the 2' position observed for 2',5'-diDMT-U (**145**). In an effort to confirm this, the reaction of $ZnBr_2$ with 2',3',5'-triDMT-U (**150**) was performed. The product was first prepared by treating uridine (**120**) with DMTCl in pyridine at 70°C. However, yields were low with this procedure (ca 40%) and as a result, the purification of the product proved difficult. A procedure developed by Ogilvie et al.[236] for selective tritylation of ribonucleosides was adapted to the preparation of the tritritylated derivative. A solution of uridine in anhydrous THF was treated with the chloride in the presence of silver nitrate and pyridine and heated to 60°C for 6 hours (Scheme LXIII). The desired product (**150**) was isolated in 87% yield. The ^1H NMR spectrum of the product revealed the considerable downfield shift of the H-1' signal which becomes obscured by the trityl signals in the 6.7 ppm range.

During the initial treatment of **150** with $ZnBr_2$, moisture was inadvertently introduced to the reaction. As a result, HBr was formed and indiscriminate detritylation occurred. A mixture of the various tritylated

Scheme XLIII



150 151

isomers was obtained. The procedure was repeated at 0°C with 10 equivalents of $ZnBr_2$ but TLC monitoring revealed only unreacted starting material. The solution was warmed to room temperature and the quantity of $ZnBr_2$ was gradually increased to 30 equivalents. The reaction was stirred overnight at room temperature and TLC monitoring at this time revealed the presence of approximately 50% of a slower moving tritylated material. This was determined to be the 2',3'-diDMT isomer and was isolated in 11% yield. Starting material was recovered in 88% yield. TLC monitoring had revealed a greater proportion of product relative to starting material than was actually obtained. This observation seemed to indicate a tendency to reaction reversal which does not occur on silica gel TLC plates. The reaction was repeated with heating to 40°C in the presence of 30 equivalents of $ZnBr_2$. This was increased to 50 equivalents and after a total reaction time of 4 hours, complete conversion of the starting material (**150**) to 2',3'-diDMT-U (**151**) was apparent by TLC. In an attempt to inhibit reaction reversal, activated silica gel was added as part of the work-up procedure. This was not effective however as 2',3',5'-triDMT-U was recovered in 62% yield and the product (**151**), obtained in only 19% yield. These results indicate that selective 5'-detritylation of ribonucleosides can be achieved with $ZnBr_2$ as long as both secondary hydroxyl functions are protected.

The ^1H NMR spectra of the tritylated uridine derivatives were recorded in deuterated nitromethane in an effort to ascertain the possible role of substituent orientation on the selectivity of the $ZnBr_2$ reaction. $^2J_{H1',H2'}$ values, which are characteristic of sugar ring conformation, are listed in Table XXVIII. A smaller coupling constant indicates predominance of the N conformation (C-3'endo) whereas a large value is characteristic of the S form (C-2'endo). The results are not very informative as both the 2',5'-diDMT-U and the 2',3',5'-triDMT-U are shown to exist predominantly in the S conformation in nitromethane. Nevertheless, detritylation occurs

TABLE XXVIII

Coupling Constants ($J_{1',2'}$) of the Tritylated Uridine Derivatives in Nitromethane-d3

Product	#	J	Product	#	J
2'5'DMT	145	7.8	5'DMT	147	7.3
3'5'DMT	146	4.4	2'3'5'DMT	150	8.3
2'DMT	129	3.9	2'3'DMT	151	7.8

J reported in Hz.

exclusively in the 2' position of the former molecule and in the 5' position of the latter. Hence, selectivity does not seem to be related to conformation but solely to the presence of an easily accessible chelation site. It appears that when both secondary hydroxyl groups (2' and 3') are protected, the steric crowding at this site is such that complexation of $ZnBr_2$ is only possible at the more accessible 5' position.

4.5 Conclusion

While the 2,4-dinitrobenzenesulfenyl group has proved excellent for the protection of base exocyclic amine functions [289], its use as a 5'-hydroxyl protecting group presents some limitations. The sulfenylation reaction is not selective and the purification of the products is laborious due to their excessive streaking during chromatographic separation. Because of these difficulties, prior protection of the secondary hydroxyl groups and protection of base exocyclic amine functions are necessary in order to obtain acceptable yields of 5' derivatised nucleosides. Furthermore, the yields of desulfenylation reactions were low, ranging from 60% for deprotection of a nucleoside to 45% for that of a dinucleotide. Although below acceptable limits for solution syntheses, these yields might be improved by use of solid support procedures. In addition, a certain proportion of product loss is attributable to the sensitivity of the trityl groups to acidic conditions. The risk of detritylation renders the addition of triethylamine necessary during removal of the dinitrobenzenesulfenyl group. The β-cyanoethyl phosphorus protecting group was found to be stable to the deprotection conditions for a 30 minute period. Nevertheless, the presence of triethylamine during removal of the nitrobenzenesulfenyl group presents a problem as this procedure must be repeated with each nucleoside addition. Moreover, to prevent detritylation of the dinucleotide prepared, it proved necessary to add traces of base to the elution solvents during chromatographic separation. As pyridine increased the problems associated with the purification of dinitrobenzenesulfenylated products, triethylamine was used. It is difficult to estimate product losses due to decyanoethylation during chromatography. However, this

problem could be eliminated by solid support methods as purification is not performed after each deprotection reaction. The difficulties encountered during purification and removal of the dinitrobenzenesulfenyl group might have been minimised by using a function other than the trityl group for 2'-hydroxyl protection. The less sensitive MMT group or a non acid labile function might have proved more effective.

The DNBS group appears to be more suited to the protection of the 2'-hydroxyl group of ribonucleosides or the base NH positions as suggested by Chattopadhyaya et al.[289]. As previously noted, the DNBS function is stable to nucleotide condensation conditions and is removed in neutral solutions. But, as in the case of silyl protecting groups, removal of the sulfenyl group following each nucleoside addition is not feasible. These properties render the DNBS function more adaptable to blocking positions which require permanent protection throughout nucleotide synthesis.

The selectivity obtained when treating a ditritylated ribonucleoside with zinc bromide differed from that reported for the reaction of deoxyribonucleoside derivatives. In the presence of a free 3'-hydroxyl group, the 2'-trityl group is removed in preference to the 5' group. This reaction is not very useful as selective 5'-tritylation of ribonucleosides is easily achieved by direct treatment with trityl chloride. The reaction of a tritritylated ribonucleoside with zinc bromide proceeded in the same manner as that of deoxyribonucleosides: the 5'-trityl group was selectively cleaved. However, the steric crowding of such molecules rendered detritylation difficult. A large excess of reagent was needed and heating of the reaction was also required. Furthermore, reaction reversal seemed to occur upon work-up and the yields obtained were low.

Contributions to Knowledge

Several analogues of the antiviral agent BIOLF-62 were prepared for testing against viruses of the herpes family. BIOLF-62 is an acyclonucleoside which has shown remarkable activity against such pathogens. The analogues were made by coupling various heterocyclic bases to the side chain of BIOLF-62. The bases were selected for their known biological activity or for their resemblance to chemotherapeutic agents. Although the acyclic analogues prepared were not toxic to host cells, none of the derivatives proved effective in inhibiting viral multiplication.

Dimethoxytritylated and t-butyldimethylsilylated derivatives of the antiviral agent arabinoadenosine were prepared for eventual incorporation into a nucleotide. By this means, drawbacks to the use of arabinoadenosine as a therapeutic drug (lack of solubility, toxicity to host cells and *in vivo* deactivation by cellular adenosine deaminase) might be eliminated. The 2'- or 3'-hydroxyl groups of arabinoadenosine were selectively protected using silver nitrate/base systems developed in this laboratory [236, 246]. The products were fully characterised by ^{1}H as well as ^{13}C NMR.

^{29}Si INEPT NMR was applied to the characterisation of nucleosides protected with silyl groups. This pulse sequence allows the signal enhancement of an insensitive nucleus, like ^{29}Si. The enhancement is ensured by transfer of polarisation from another nucleus, such as hydrogen, which possesses a large Boltzman population difference. By this technique, it was determined that 2'- and 3'-silyl isomers can be easily distinguished on the basis of their respective chemical shifts. As no such differentiation was possible for disilylated ribonucleosides, the silicon signals of

these derivatives were unambiguously assigned by the ^{29}Si-^{1}H Hetero-correlation technique.

The 2,4-dinitrobenzenesulfenyl function (DNBS) was investigated for the protection of the 5'-hydroxyl group of ribonucleosides. Nitrobenzene-sulfenyl groups are stable to both acidic and basic conditions and can be removed in neutral solution. These properties should render the DNBS group suitable for use with the phosphodichloridite nucleoside coupling proce-dure. The mild conditions under which the group is removed should make it particularly interesting for the protection of the 5' position. The ease of removal of the DNBS group as well as its compatibility with the phos-phodichloridite coupling conditions were confirmed. A uridine dinucleotide (UpU) was prepared using the DNBS group for 5'-hydroxyl protection and the dimethoxytrityl function in the 2' position. The use of the DNBS group was found to present some drawbacks. Selective protection of the 5' position proved difficult and purification of the DNBS derivatives was laborious. The DNBS group appears more suited to the protection of base exocyclic amine groups as suggested by Chattopadhyaya [289], or to the permanent protection of the 2' position of ribonucleosides.

Finally, the reactions of tritylated ribonucleosides with zinc bro-mide were investigated. While in the deoxyribonucleoside series, a trityl group in the 5' position is cleaved in preference to that protecting the secondary hydroxyl group (3'), the reverse selectivity was observed for a ditritylated ribonucleoside. This was attributed to the presence of an alternate zinc bromide chelation site between the oxygen atoms in the 2' and 3' positions. This site may compete with that between the 5' and sugar ring oxygen atoms which favours detritylation in the 5' position. Reaction

of a tritritylated ribonucleoside resulted in selective cleavage of the 5'-trityl group as in the case of deoxyribonucleosides. The steric crowding of this molecule seems to interfere with complexation of zinc bromide between 2' and 3' positions.

CHAPTER FIVE

Experimental Section

5.0 General Methods

5.0.1 Reagents and Chemicals

Reagent grade tetrahydrofuran (THF) was first kept over potassium hydroxide for several days then refluxed over sodium and benzophenone (BDH Chemicals), under nitrogen, until the development of a purple colour. Solvent was collected from the still as needed.

Reagent grade N,N-Dimethylformamide (DMF), 2,4,6-trimethylpyridine and dimethylsulfoxide (DMSO) were stirred with mild heating over calcium hydride for 12 hours then vacuum distilled and stored over type 4Å molecular sieves.

Reagent grade pyridine, dioxane and nitromethane were stirred over calcium hydroxide for 12 hours then fractionally distilled and stored over 4Å molecular sieves.

Reagent grade dichloroethane and dichloromethane were distilled over phosphorus pentoxide then stored over 4Å molecular sieves.

The ethanol used was 95%. All other solvents were reagent grade and used without further purification.

The unprotected nucleosides, adenosine, cytidine, guanosine, uridine, thymidine and arabinoadenosine were obtained from Sigma or Boehringer Mannheim.

The benzoyl, monomethoxytrityl and dimethoxytrityl derivatives were prepared according to literature procedures [79, 90]. The t-butyldimethylsilyl derivatives were prepared by methods developed in our laboratory [123, 236]

Tert-butyldimethylsilyl chloride (Aldrich or Sigma), triisopropylsilyl chloride (Aldrich), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (Al-

drich), dimethoxytrityl chloride (Sigma), monomethoxytrityl chloride (Sigma) and 2,4-dinitrobenzenesulfenyl chloride (Aldrich) were stored in the refrigerator and allowed to warm to room temperature in a dessicator prior to use.

5.0.2 Product isolation and analyses

Thin-layer chromatography (TLC) was performed by the ascending technique on Merck Kieselgel 60 F_{254} (.2 mm) or Cellulose F (.1 mm). Thick layer chromatography was done on 20 cm^2 glass plates coated with 1 mm of silica gel (Merck Kieselgel 60 GF_{254}). Merck Kieselgel 60 (230-400 Mesh) was used for column chromatography.

Nucleosides and their derivatives were visualised on silica and cellulose with an ultraviolet light source (Mineralight, 254 nm). Monomethoxytritylated and dimethoxytritylated compounds were further detected by spraying with a 10% perchloric acid solution. Non UV absorbing material was revealed by exposure to iodine vapours.

Products were recovered from silica gel and cellulose plates by elution with organic solvents (methanol or ethanol/ethyl acetate) or water. The organic solvents were removed under reduced pressure on a Büchi rotary evaporator and the water was removed in a Savant Speed-Vac.

Melting points, reported uncorrected, were determined on a Fisher-Johns apparatus. Ultraviolet spectra were recorded on a Varian Cary 17, Varian Cary 210 or Hewlett Packard 8451A spectrophotometer using ethanolic or aqueous solutions.

Nuclear magnetic resonance spectra of ^{1}H, ^{13}C, ^{29}Si and ^{31}P were obtained on either a Brüker WH-90 (90 MHz), a Brüker WH-400 (400 MHz), a Varian XL-200 (200 MHz) or a Varian XL-300 (300 MHz) spectrometer. These were recorded in either 5mm or 10 mm tubes within a temperature range of 20°C $\pm$3°. All chemical shifts are reported in ppm downfield from tetramethylsilane (TMS) with the exception of the negative phosphorus shifts which are reported in ppm upfield from 85% phosphoric acid, used as an external reference. On the basis of the experimental parameters used, the digital resolution obtained for ^{1}H NMR is of 0.001 ppm and of 0.03 ppm for ^{13}C, ^{29}Si and ^{31}P NMR. The error is estimated to be very near the digital

resolution for the ^{1}H and ^{13}C peak positions which are reproducible to within a range of ± 0.01 and ± 0.05 ppm respectively. However, due to the concentration dependance of ^{29}Si peak positions and the use of an external reference for ^{31}P NMR, the chemical shifts obtained for these nuclei are only reproducible to within ± 0.1 ppm

Electron impact (EI) mass spectra were obtained on a DuPont Instruments 21-492-B mass spectrometer and the fast atom bombardment (FAB) spectrum was obtained from a glycerol matrix on a Vacuum Generators ZAB-2F instrument.

Elemental analyses were performed by Canadian Microanalytical Service Ltd., Vancouver, B.C.. Samples were prepared by crystallisation from methanol, ethanol or water and dried by heating (68°C) under reduced pressure.

High pressure liquid chromatography analyses of enzyme degradation assays were performed on a Spectra-Physics SP8000 instrument equipped with a UV detector (SP8200, 254 nm) and a SP4000 Chromatography data system. Reversed phase Whatman ODS-2, 10 u particles (Chromatographic Specialties, Brockville, Ontario) served as column support. HPLC grade ammonium acetate was used and trace amounts of sodium azide (0.001% by wt/volume) were added to aqueous buffers to prevent bacterial growth. All buffers were filtered through a 0.45 filter (Millipore Corporation, Bedford, MA.) prior to use.

Biological testing was performed by Dr. Kendall O. Smith at the University of Texas Health Science Center or by Bristol Research Laboratories. Compounds were assayed <u>in vitro</u> against strains of herpes simplex viruses (HSV-I and II) grown in vero or human fetal lung (HFL) cell monolayers.

5.1 Preparation of Novel Heterocyclic-Ring Analogues of BIOLF-62 : (Chapter 1)

5.1.1 1,3-Dibenzyloxy-2-Chloromethoxypropane 66

1,3-Dibenzyloxy-2-propanol 65 was prepared in 97% yield according to a procedure developed in this laboratory [144] with minor modifications. Under a constant stream of argon, sodium hydride (40 g) was added portion-wise to benzyl alcohol (500 mL, 4.83 mol.) over a 30 to 45 minute period instead of over 15 minutes. Following the addition of 1,3-dichloropropan-2-ol (26 g, 0.20 mol), the reaction was heated overnight at 90° instead of 100°C. The product was vacuum distilled (b.p. 174°C, 0.080 KPa; lit. b.p. 195-210°C, 3 torr). ^{1}H NMR (200 MHz, $CDCl_3$): 7.28 (10H, s, Ph); 4.48 (4H, s, $\underline{CH_2}Ph$); 3.90 (1H, qi, CH); 3.50 (4H, m, $\underline{CH_2}CH$); 2.92 (1H, m, OH).

In the preparation of 1,3-Dibenzyloxy-2-chloromethoxypropane 66, the procedure outlined by Ogilvie et al.[144] was adhered to with the exception of the length of time HCl gas was bubbled through a solution of 65 and paraformaldehyde in dichloroethane (3 hours versus 6). Also, the gas stream was passed through a tube containing calcium chloride before entering the reaction flask to avoid introduction of moisture. The product, a clear oil, was obtained in 86% yield (unreacted alcohol (48) was estimated at 14% by ^{1}H NMR) and used without further purification (reported yield: 91% by weight). ^{1}H NMR (200 MHz, $CDCl_3$): 7.41 (10H, s, Ph); 5.74 (2H, s, CH_2Cl); 4.61 (4H, s, $\underline{CH_2}Ph$); 4.25 (1H, qi, CH); 3.70 (4H, m, $\underline{CH_2}CH$).

5.1.2 2-Methylthiomethyl-1,3-Dibenzyloxypropyl ether 85

A modification of the procedure developed by Pojer et al.[168] for the preparation of methylthiomethyl ethers was used. A mixture of alcohol 65 (13.9 g, 51 mmol.), anhydrous dimethylsulfoxide (165 mL), glacial acetic acid (33 mL) and acetic anhydride (109 mL) were stirred together at R.T. for 48 hours then poured over a cold solution of sodium carbonate (0-5°C) and extracted into dichloromethane. The product was purified by column chromatography on silica gel (5.5 cm diameter, 320 g) using EtOAc/Hexanes (2:8) then distilled on a Kugelrohr apparatus (b.p. 155-159°C, 0.027 KPa) to yield 47% of 85 as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): 7.33

(10H, s, Ph); 4.81 (2H, s, CH_2); 4.55 (4H, s, $\underline{CH_2}^{Ph}$); 4.13 (1H, m, CH); 3.64 (4H, m, ^{CH_2}CH); 2.14 (3H, s, $\underline{CH_3}S$).

5.1.3 Direct Coupling Procedures with Chloromethyl ether **66**

The methods outlined by Ogilvie et al.[144, 295] were applied with some modifications. The products were purified on silica gel plates (100 mg material/plate) or by column chromatography (20-25 g silica/g of material).

a) The heterocyclic base (3 eq.) was first dissolved in anhydrous DMF (1-2 mL/mmol. of base) and the solution was cooled to 5°C. The chloromethyl ether **66** (1 eq.) was added and the reaction stirred for 2 hours at 5°C then overnight at R.T.. The DMF was eliminated by co-evaporation with ethanol and the residue was taken up in dichloromethane, washed with a 5% sodium bicarbonate solution then concentrated under reduced pressure.

b) To a solution of the heterocyclic base (1 eq.) and triethylamine (1.2 eq.) in anhydrous DMF or THF (1-2 mL/mmol.) was added chloromethylether **66** (1.2 eq.). The reaction was stirred 12 hours at R.T.. Following filtration of the triethylamine hydrochloride produced, the mixture was concentrated to an oil under reduced pressure.

5.1.4 Hexamethyldisilazane Mediated Coupling Procedures

These procedures, modified from that reported by Ogilvie et al.[164-166], were performed under argon atmosphere. Products were purified as described in 5.1.3.

a) Chloromethyl Ether **66** Coupling

In a three-necked flask equipped with an argon entry port and a condenser, the heterocyclic base (1.1 eq.), ammonium sulfate (2 mg/mmol. of base) and hexamethyldisilazane (HMDS) (12 eq., 1.3 mL/mmol. of base) were refluxed until the base was completely dissolved (20 min. to 3 hours). The excess HMDS was distilled of under reduced pressure with mild heating (40°C) and residual silazane was removed on a vacuum pump (30-40 min.). At this time, the mixture appeared as either a colourless oil or a white

solid. Argon atmosphere was restored and the flask was equipped with a septum. The residue was dissolved in anhydrous THF added via syringe then tetrabutylammonium iodide (240 mg/mmol. of base) in THF (0.3 mL/100 mg) and chloromethylether **66** (1 eq.) in THF (1 mL/mmol. + rinse (2 x .25 mL)) were added. The reaction was stirred overnight at R.T. then taken up in dichloromethane, washed with a 5% sodium bicarbonate solution and concentrated to an oil under reduced pressure.

b) Methylthiomethyl Ether **85** Coupling

The reaction was first executed as in a) then, following the dissolving of the residue in THF, an iodine solution (254 mg/mmol. of base) in THF (0.3 mL/100 mg I_2) and a methylthiomethyl ether **85** solution in THF (1 eq.; 0.6 mL/mmol. + rinse (2 x .25 mL)) were added. The mixture was stirred at room temperature then gently refluxed for 6 to 8 hours. After cooling, a 5% sodium sulfite solution was added dropwise with stirring until the iodine colour no longer persisted. The product was extracted into dichloromethane, washed with a 10% sodium chloride solution then concentrated to an oil.

5.1.5 Bis(tri-n-Butyltin) Oxide Coupling Procedure

A procedure developed by Balasubramanian *et al.*[169], was used with slight modifications. A mixture of the heterocyclic base (1.1 eq.) and bis(tri-n-butyltin) oxide (0.5 eq.) in distilled petroleum ether (30-60°C or 90-120°C) was refluxed for 3 hours. The water produced was removed with a Dean-Stark apparatus. Chloromethyl ether **66** (1 eq.) was added and the reaction was refluxed overnight. After cooling, water was added and the mixture was extracted 4 times into dichloromethane. The combined extracts were washed with a dilute solution of acetic acid (3%) then concentrated to an oil. Special precautions were taken in handling the tin oxide: the wearing of gloves, the soaking of used glassware in an ethanolic KOH bath, the disposal of used silica gel in sealed plastic bags and the separate disposal of waste solvents.

5.1.6 Removal of the Benzyl groups

a) Catalytic Transfer Hydrogenation

In a modification of the procedure described by Ogilvie et al.[296], palladium oxide (100 mg/mmol.) and cyclohexene (1.2 mL/mmol.) were added to a solution of the dibenzyl compound in absolute ethanol (4mL/mmol.). The reaction was refluxed for 1½-3 hours and monitored by TLC. After cooling, the mixture was filtered over celite and the solid washed with ethanol. The filtrate was concentrated to an oil.

b) Boron Trichloride Procedure

This procedure was modified from that published by Seela et al.[141]. The dibenzyl derivative was dissolved in anhydrous dichloromethane (50 mL/mmol.), cooled to -78°C (dry ice/isoPrOH) and treated with boron trichloride (8 eq.) from a freshly opened bottle (1M BCl_3 in CH_2Cl_2, Aldrich). The reaction was stirred 2-3 hours under argon atmosphere then monitored by TLC (aliquots were hydrolysed and neutralised); at this time, if starting material was still apparent, more BCl_3 (3 eq.) was added and the reaction allowed to proceed 1 more hour. While still at low temperature, methanol (5 mL/mmol.) was added and the reaction was brought to pH 7 by addition of triethylamine. Following concentration under reduced pressure, the residue was taken up in dichloromethane and filtered to remove the triethylamine hydrochloride produced. The filtrate was then concentrated to an oil under reduced pressure.

5.1.7 1-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]imidazole **68**

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]imidazole **67** was prepared by method 5.1.3a without the initial cooling period and purified on silica gel plates using $Et_2O/CH_2Cl_2/EtOH$ (10:4:1) or by column chromatography with CH_2Cl_2/EtOH (9:1). The product, a yellow oil, was obtained in 67% yield. R_f [Et_2O/CH_2Cl_2/EtOH (10:4:1)] = 0.40.

The final compound was prepared by catalytic transfer hydrogenation (5.1.6a) and was obtained in 86% yield after purification by column chromatography on silica gel using CH_2Cl_2/MeOH (9.5:0.5). The yellowish oil isolated formed waxy crystals after three weeks in methanol/H_2O. These

were repeatedly washed with cold diethyl ether to yield white crystals. Anal. calculated for $C_7H_{12}O_3N_2(+\frac{1}{2}\ H_2O)$: C, 48.83 (46.40); H, 7.02 (7.23); N, 16.27 (15.46). Found: C, 46.26; H, 6.89; N, 14.71. The physical and NMR properties of the analogues prepared are summarised in Tables II to VIII.

5.1.8 1-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]benzimidazole 70

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]benzimidazole 69 was prepared by procedures 5.1.3a, 5.1.4a and 5.1.5 in yields of 83%, 60% and 67% respectively. Purification was done on silica gel plates using Et_2O/ CH_2Cl_2/EtOH (10:4:1) or by column chromatography with CH_2Cl_2/EtOH (9.9:0.1) to give a colourless oil. R_f [CH_2Cl_2/EtOH (9.5:0.5) = 0.14. ^1H NMR (200 MHz, $CDCl_3$): 7.89 (1H, s, H-2); 7.72 (1H, m, H-4); 7.48 (1H, m, H-7); 7.23 (12H, m, H-5, H-6, Ph); 5.65 (2H, s, CH_2N); 4.37 (4H, s, $\underline{CH_2}Ph$); 3.77 (1H, m, CH); 3.45 (4H, m, $\underline{CH_2}CH$). ^{13}C NMR (WH-90, $CDCl_3$): 1147.00 (C-2); 146.90 (C-8); 141.41 (C-9); 126.95, 126.20 (C-5, C-6); 123.77 (C-4); 114.06 (C-7); 79.75 (C-3'); 76.63 (C-1'); 74.53 ($\underline{CH_2}Ph$); 71.99 (C-4').

The benzyl groups were removed by catalytic transfer hydrogenation (5.1.6a). The product was purified on silica gel plates [CH_2Cl_2/EtOH (9.9:0.1)] or by column chromatography [CH_2Cl_2/EtOH (9.5:0.5)] to yield 66% of a colourless oil which readily crystallised from methanol. Anal. calculated for $C_{11}H_{14}O_3N_2$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.10; H, 6.32; N, 12.33.

5.1.9 1-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]-7-Azaindole 72

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-7-azaindole 71 was prepared by procedure 5.1.3b in DMF and THF in 60% and 70% yields respectively. By repeating the reaction in a more dilute solution of THF (5 mL/mmol.) the yield was increased to 93%. The product was purified to a yellow oil on silica gel plates or by column chromatography using Et_2O/ Hexanes (1:1). R_f [Et_2O/ Hexanes (1:1)] = 0.48. ^1H NMR (200 MHz, $CDCl_3$): 8.34 (1H, d, J = 4.1 Hz, H-6); 7.83 (1H, d, J = 7.8 Hz, H-4); 7.33 (1H, d, J = 3.6 Hz, H-2); 7.24 (10H, m, Ph); 7.03 (1H, dd, J = 4.7, 7.8 Hz, H-5); 6.45 (2H, d, J = 3.5 Hz, H-3); 5.85 (2H, s, CH_2N); 4.39 (4H, s, $\underline{CH_2}Ph$);

4.03 (1H, m, CH); 3.49 (4H, m, $\underline{CH_2}CH$). ^{13}C NMR (WH-90, CD_3OD): 148.83 (C-2); 143.63 (C-8); 130.37 (C-6); 129.85 (C-5); 122.64 (C-4); 117.51 (C-9); 102.10 (C-3); 77.53 (C-3'); 74.35 (C-1'), 74.02 ($\underline{CH_2}Ph$); 71.10 (C-4').

Treatment of **71** with BCl_3 (5.1.6b) afforded the product in 64% yield after column chromatography on silica gel using EtOAc/MeOH (9:1). The colourless oil thus obtained crystallised readily from methanol. Anal. calculated for $C_{11}H_{14}O_3N_2$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.17; H, 6.39; N, 12.53.

5.1.10 1-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]isatin **74**

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]isatin **73** was prepared in THF according to procedure 5.1.3b with heating overnight at 40°C. The product was purified on silica gel plates [EtOAc/Hexanes (1:2)] or by column chromatography [EtOAc/Hexanes (1:1)] and obtained in 88% yield as a yellow oil. R_f [EtOAc/Hexanes (1:1)] = 0.66. ^1H NMR (200 MHz, $CDCl_3$): 7.55 (1H, m, J = 7.5 Hz, H-6); 7.29 (13H, m, H-4, H-5, H-7, Ph); 5.32 (2H, s, CH_2N); 4.43 (4H, 2s, $\underline{CH_2}Ph$); 3.56 (1H, m, CH); 3.52 (4H, m, $\underline{CH_2}CH$). ^{13}C NMR (WH-90, CD_3OD): 184.11 (C-3); 160.27 (C-2); 151.82 (C-8); 139.67 (C-6); 128.62, 125.45 (C-4, C-5); 119.00 (C-9); 112.96 (C-7); 78.57 (C-3'); 74.28 ($\underline{CH_2}Ph$); 72.72 (C-1'); 70.71 (C-4').

The benzyl groups were removed by methods 5.1.6a and 5.1.6b in yields of 35% and 76% respectively. Purification was effected on silica gel plates or by column chromatography using CH_2Cl_2/MeOH (9.5:0.5). The yellow oil obtained crystallised upon standing to a waxy solid which was vigorously stirred in diethyl ether for 12 hours to produce bright orange crystals. Anal. calculated for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.22; N, 5.58. Found: C, 56.85; H, 5.21; N, 5.38.

5.1.11 9-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]-6-Methoxypurine **81**

9-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-6-methoxypurine **80** was first prepared in 56% yield by method 5.1.3b in DMF. The 7-isomer **82** was recovered in 38% yield. When the coupling was repeated using procedure 5.1.4b, the desired 9-isomer was favoured over the other (65% to 6.5%) although the overall yield was inferior. The product was purified to a

colourless oil on silica gel plates [Et_2O/CH_2Cl_2/EtOH (10:4:0.5)] or by column chromatography [Et_2O/CH_2Cl_2/EtOH (10;4;10)]. 9-isomer: R_f [idem (10:4:1)] = 0.51; UV (EtOH): λ_{max} = 247 nm; λ_{min} = 223 nm. 7-isomer: R_f [idem (10:4:1)] = 0.42; UV (EtOH): λ_{max} = 232, 258 nm.

Treatment of the 9-isomer with BCl_3 (5.1.6b) followed by purification on silica gel plates [EtOAc/$CHCl_3$/EtOH (5:4:1)] afforded the final compound in 89% yield as a yellow oil which crystallised from methanol. M.S. (FAB, 7KV, 26°C) m/z: 255.2 [MH^+]; 239.3 [M^+-15].

5.1.12 1-[[2-Benzyloxy-1-(Benzyloxymethyl)ethoxy]methyl]indole **86**

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]indole was prepared according to a method described by Sundberg and Russell [170]. Sodium methylsulfinylmethide was generated by the method described by Corey and Chaykovsky [167]. Sodium hydride (320 mg of a 60% suspension in oil, 2.0 mmol.) was washed with petroleum ether then added to anhydrous DMSO (5 mL) in a three necked flask equipped with an argon entry port, a condenser and drying tube and fitted with a septum. The mixture was heated to 75-85°C for 45 minutes then cooled to 0-5°C. A solution of indole (468 mg, 4 mmol.) in anhydrous THF (3 mL) was added dropwise via syringe. The reaction was allowed to warm to R.T. and was stirred 30 minutes before cooling once more to 0-5°C. A solution of **66** (1.81 g, 4.5 mmol.) in anhydrous THF (2 mL) was then added dropwise via syringe and the reaction was stirred 1 hour at room temperature. The mixture was poured into a dilute sodium carbonate solution (10 mL) and extracted several times into dichloromethane which was then dried over sodium sulfate and concentrated under reduced pressure. The product was purified by column chromatography on silica gel using Et_2O/Hexanes (1:3). The product was obtained in 82.8% yield as a colourless oil which was stored at 0°C under inert atmosphere. The oil gradually darkens to black when exposed to air and left standing overnight at room temperature. R_f [EtO_2/Hexanes (1:1)] = 0.58; UV (EtOH): λ_{max} = 266 nm; λ_{min} = 240 nm. ^1H NMR (200 MHz, $CDCl_3$): 7.97 (1H, d, J = 8.0 Hz, H-7); 7.57 (1H, d, J = 7.8 Hz, H-4); 7.39 (13H, m, Ph, H-2, H-5, H-6); 6.57 (1H, d, J = 2.8 Hz, H-3); 5.76 (2H, s, CH_2N); 4.61 (4H, s, $\underline{CH_2}Ph$); 4.19 (1H, m, CH); 3.69 (4H, m, $\underline{CH_2}CH$). ^{13}C NMR (XL-300, acetone-

d6): 138.46 (C-8); 129.12, 128.34 (C-9, C-2); 121.58 (C-5); 120.47 (C-4); 119.78 (C-6); 110.19 (C-7); 101.91 (C-3); 75.31 (C-3'); 75.16 (C-1'); 72.63 ($\underline{CH_2}$Ph); 70.41 (C-4').

5.1.13 2-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]indazole **88**

2-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]indazole **87** was prepared in 94% yield by procedure 5.1.3b in THF. The product, a yellow oil was purified on silica gel plates or by column chromatography using EtOAc/Hexanes (1:1). R_f [EtOAc/Hexanes (1:1)] = 0.43. ^{1}H NMR (200 MHz, $CDCl_3$): 8.14 (1H, s, H-3); 7.75 (1H, dd, J = 8.3, 0.9 Hz, H-7); 7.64 (1H, dd, J = 8.5, 0.9 Hz, H-4); 7.30 (11H, m, H-5, Ph); 7.23 (1H, m, J = 8.8, 1.0 Hz, H-6); 5.92 (2H, s, CH_2N); 4.46 (4H, s, CH_2Ph); 4.08 (1H, m CH); 3.55 (4H, dd, J = 5.1 Hz, $\underline{CH_2}CH$). ^{13}C NMR (WH-90, $CDCl_3$): 155.79 (C-8); 139.56 (C-3); 126.85 (C-4); 125.78 (C-9); 121.31, 120.60 (C-5, C-6); 118.33 (C-7); 84.70 (C-1'); 80.02 (C-3'); 74.69 ($\underline{CH_2}$Ph); 71.05 (C-4').

The benzyl groups were removed by treatment with BCl_3 (5.1.6b). Purification by column chromatography on silica gel [EtOAc/MeOH (9:1)] yielded 89% of **88** as a yellow oil. Crystallisation attempts from a variety of solvents (MeOH, CH_2Cl_2/MeOH/Hexanes, CH_2Cl_2/MeOH/Pet. ether, H_2O) were unsuccessful. Anal. calculated for $C_{11}H_{14}O_3N_2$ (+½ H_2O): C, 59.45 (57.13); H, 6.35 (6.54); N, 12.60 (12.11). Found: C, 57.93; H, 6.38; N, 12.01.

5.1.14 1-[[2-Hydroxy-1-(Hydroxymethyl)ethoxy]methyl]-4-Nitroimidazole **90**

1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-4-nitroimidazole **89** was prepared by procedure 5.1.3b in THF and was purified on silica gel plates or by column chromatography [CH_2Cl_2/MeOH (9.7:0.3)]. The product, a yellow oil, was obtained in 95% yield. R_f [CH_2Cl_2/EtOH (9.5:0.5)] = 0.56.

Treatment of **89** with BCl_3 (5.1.6b) and purification by column chromatography on silica gel using EtOAc/MeOH (9:1) afforded the final product in 74% yield. The oil was crystallised to white needles from methanol/H_2O. Anal. calculated for $C_7H_{11}O_5N_3$ (+½ H_2O): C, 38.71 (37.17); H, 5.11 (5.35); N, 19.35 (18.58). Found: C, 37.55; H, 4.93; N, 18.20.

5.1.15 1-[[2-Benzyloxy-1-(Benzyloxymethyl)ethoxy]methyl]-8-Azaadenine 91

Procedure 5.1.4b was used to effect the coupling of 8-azaadenine with 66. Both the desired 9-isomer (91) and the 8-isomer (92) were formed and were purified by column chromatography [Et_2O/CH_2Cl_2/EtOH (10:4:1)]. Compounds 91 and 92 were isolated as colourless oils in yields of 45% and 11% respectively. 9-isomer: R_f [idem (10:4:1)] = 0.76; UV (EtOH): λ_{max} = 280 nm; λ_{min} = 237 nm. 8-isomer: R_f [idem (10:4:1)] = 0.46; UV (EtOH): λ_{max} = 296 nm; λ_{min} = 265 nm.

5.2 Preparation of Protected Arabinoadenosine Nucleosides : (Chapter 2)

5.2.1 5'-Dimethoxytritylarabinoadenosine 99

Dimethoxytrityl chloride (DMTCl) (2.54 g, 4.60 mmol.) was added in equal portions (400 mg) at regular intervals over a 6 hour period to a stirred solution of araA (1 g, 3.8 mmol.) in anhydrous pyridine-DMF (1:1, 5 mL/mmol.) maintained at 0°C. The reaction was stirred another 4 hours at 0°C and monitored by TLC [$CHCl_3$/EtOH, (7:3)]. Although some starting material was still present, the mixture was poured onto ice water (500 mL) and the solid product was collected by filtration then purified by successive washings with diethyl ether or by column chromatography on silica gel (5 cm column, 100 g) using $CHCl_3$/MeOH (9:1) to yield 5'-DMTaraA as a white powder. Yields, physical properties and other NMR data for this and the following products are collected in Tables X-XIV.

5.2.2 5-t-Butyldimethylsilylarabinoadenosine 100

A modification of the method outlined by Baker et al.[218] was used. Imidazole (76 mg, 1.11 mmol.) and t-butyldimethylsilyl chloride (TBDMSCl) (84 mg, 0.56 mmol.) were added to a solution of araA (100 mg, 0.37 mmol.) in anhydrous DMF. After stirring 2 hours at R.T. with regular monitoring by TLC [$CHCl_3$/EtOH (8:2)], the mixture was poured onto ice water (50 mL) and the resulting white solid was collected by filtration. The product was purified by column chromatography on silica gel (2 cm diameter, 16 g) using $CHCl_3$/MeOH (9.5:0.5) to yield a white powder. ^1H NMR (400 MHz, dmso-d6): 7.37 (2H, m, NH_2); 6.37 (1H, d, J = 5.32 Hz, H-1'); 5.77 (1H, d, J = 5.06 Hz, OH-2'); 5.68 (1H, d, J = 5.06 Hz, OH-3'). Anal. calculated for $C_{16}H_{27}O_4N_5Si$: C, 50.37; H, 7.13; N, 18.36. Found : C, 49.97; H, 6.88; N, 17.80.

5.2.3 2',5'-di-t-Butyldimethylsilylarabinoadenosine 101

The product was prepared by Dr. Hosein Hakimelahi [243] on a 0.56 mmol. scale. Column chromatography on silica gel (2 cm diameter, 20g) using $CHCl_3$/MeOH (9.7:0.3) yielded pure 2',5'-diTBDMSaraA as a white powder. ^1H

NMR (200 MHz, dmso-d6): 7.2 (2H, m, NH_2); 6.3 (1H, bs, H-1'); 5.6 (1H, d, OH-3'). ^{1}H NMR (400 MHz, acetone-d6): 3.62 (1H, dd, J = 9.98, 6.53 Hz, H-5'); 3.47 (1H, dd, J = 9.93, 3.74 Hz, H-5'). Anal. calculated for $C_{22}H_{41}O_4N_5Si_2$: C, 53.30; H, 8.34; N, 14.13. Found: C, 53.08; H, 8.15; N, 13.89.

5.2.4 3',5'-di-t-Butyldimethylsilylarabinoadenosine **102**

To a solution of 3-methylpyridine-N-oxide (212 mg, 1.95 mmol.) in anhydrous THF (20 mL/mmol.) were successively added silver nitrate (280 mg, 1.65 mmol.) and TBDMSCl (282 mg, 1.88 mmol.). After 1½ hours, araA (200 mg, 0.75 mmol.) was added and the reaction stirred for another 2 hours at R.T. with regular TLC monitoring [$CHCl_3$/EtOH (8:2)]. The silver chloride formed was removed by filtration and 5% sodium bicarbonate (20 mL) was added to the filtrate. Following extraction into dichloromethane and concentration, the oil obtained was purified by column chromatography on silica gel (2 cm diameter, 20 g). The $CHCl_3$/MeOH (9.7:0.3) eluting system allowed separation of the product, a white powder, from 2',5'-diTBDMSaraA. ^{1}H NMR (200 MHz, dmso-d6): 7.40 (2H, m, NH_2); 6.39 (1H, bs, H-1'); 5.91 (1H, d, OH-2'). Anal. calculated for $C_{22}H_{41}O_4N_5Si_2$ (+½ EtOH): C, 53.30 (53.25); H, 8.34 (8.55); N, 14.13 (13.50). Found: C, 53.55; H, 7.77; N, 13.16.

5.2.5 5'-Dimethoxytrityl-2'-t-Butyldimethylsilylarabinoadenosine **103**

TBDMSCl (336 mg, 2.23 mmol.) was added to anhydrous THF (20 mL/mmol. of nucleoside) containing pyridine (518 mg, 6.55 mmol.) and silver nitrate (334 mg, 2.23 mmol.). After 15 minutes, 5'-DMTaraA **99** (746 mg, 1.31 mmol.) was added and the reaction was stirred at R.T. for 2 hours with monitoring by TLC [$CHCl_3$/DMF (8:2)]. The mixture was then filtered into a 5% sodium bicarbonate solution, extracted into dichloromethane and concentrated under reduced pressure. The resulting oil was purified by column chromatography on silica gel (3 cm diameter, 40 g). The $CHCl_3$/MeOH (9:1) eluting system allowed separation of the desired product, a white powder, from the 3'-isomer. ^{1}H NMR (400 MHz, acetone-d6): 3.68 (1H, dd, J = 7.30, 9.79 Hz, H-5'); 3.44 (1H, dd, J = 9.88, 3.79 Hz, H-5").

5.2.6 5'-Dimethoxytrityl-3'-t-Butyldimethylsilylarabinoadenosine **104**

To a solution of 3-methylpyridine-N-oxide (408 mg, 3.72 mmol.) in anhydrous THF (20 mL/mmol. of nucleoside) were successively added silver nitrate (525 mg, 3.07 mmol.) and TBDMSCl (494 mg, 3.26 mmol.). After 1 hour at R.T., 5'-DMTaraA **99** (533 mg, 0.93 mmol.) was added and the reaction stirred for another hour [TLC: $CHCl_3$/DMF (8:2)]. Following filtration and extraction as previously described (5.2.5), the product was purified by column chromatography on silica gel (3.5 cm diameter, 25 g) using $CHCL_3$/MeOH (9.7:0.3) to yield a white powder. ^{1}H NMR (400 MHz, acetone-d6): 3.90 (6H, 2s, OCH_3); 3.58 (1H, m, H-5'); 3.51 (1H, m, H-5").

5.2.7 Detritylation of **103** and **104**

Pure 5'-DMT-2'-TBDMSaraA or 5'-DMT-3'-TBDMSaraA (342 mg, 0.50 mmol.) were dissolved in 80% acetic acid (10 mL) and heated to 50-60°C in a water bath for 15 minutes. As the mixture was brought to pH 7 by dropwise addition of a saturated sodium bicarbonate solution, the respective monosilylated derivatives precipitated out. These were filtered, dried then washed several times with diethyl ether.

2'-TBDMSaraA (**106**): ^{1}H NMR (200 MHz, dmso-d6): 6.42 (1H, d, J = 5.15 Hz, H-1'); 5.74 (1H, m, OH-3');5.15 (1H, m, OH-5'). Anal. calculated for $C_{16}H_{27}O_4N_5Si$ (+1 H_2O): C, 50.37 (48.10); H, 7.13 (7.32); N, 18.36 (17.53). Found: C, 47.56; H, 6.94; N, 16.77.

3'-TBDMSaraA (**107**): Anal. calculated for $C_{16}H_{27}O_4N_5Si$ (+1 EtOH): C, 50.37 (50.56); H, 7.13 (7.78); N, 18.38 (16.38). Found: C, 50.41; H, 7.17; N, 15.80.

5.2.8 2',3'-di-t-Butyldimethylsilylarabinoadenosine **108**

The product was prepared by Dr. Hosein Hakimelahi [243]. Purification by column chromatography on silica gel (30 g/mmol. of nucleoside) using $CHCl_3$/MeOH (9.7:0.3) yielded a pure white powder. ^{1}H NMR (200 MHz, dmso-d6): 6.42 (1H, d, J = 4.64 Hz, H-1'); 5.16 (1H, t, OH-5').

5.3 The Application of ^{29}Si NMR to the Characterisation of Nucleosides : (Chapter 3)

5.3.1 N-Benzoyl-5'-Monomethoxytrityl-2'-Triisopropylsilylguanosine

To a solution of N-Bz-5'-MMT-G (4.40 g, 6.67 mmol.) in anhydrous DMF (60 mL) were added imidazole (1.04 g, 15.28 mmol.) and triisopropylsilyl chloride (TiPSCl) (2.5 mL, 11.1 mmol.). The reaction was stirred overnight at R.T. then monitored by TLC [$CHCl_3$/EtOH (9.5:0.5) and $CHCl_3/Et_2O$ (1:1)]. Due to the presence of unreacted starting material, imidazole (0.21 g, 2.78 mmol.) and TiPSCl (0.5 mL, 2.1 mmol.) were again added and the reaction allowed to proceed for 10 hours at which time the DMF was co-evaporated several times with ethanol. The residue was dissolved in dichloromethane and washed with a 5% solution of sodium bicarbonate. The yellow foam obtained upon evaporation of dichloromethane was purified by column chromatography on silica gel (3 cm diameter, 130 g) using $CHCl_3/CH_2Cl_2$/TEA (9:0.5:0.5). Residual triethylamine from the collected fractions was immediately eliminated by co-evaporation with xylene in order to avoid isomerisation of the silyl groups. The recovered yields were 47% and 23% respectively of the 2' and 3' isomers as white powders.

The 3'-triisopropylsilyl nucleoside (1.3 g) was easily isomerised to a 1:1 mixture by a procedure described for the isomerisation of 3'-TBDMS nucleosides [245]. The compound was dissolved in an ammonium hydroxide/ethanol solution (2 g : 200 mL) and stirred overnight. The products which precipitated upon cooling, were collected by filtration. The recovered yield was 91% due to some debenzoylation. Selective crystallisation of the 2' isomer upon standing in the ammonium hydroxide solution has been observed [297].

N-Bz-5'-MMT-2'-TiPS-G: m.p. 123°C; R_f [$CHCl_3$:Et_2O (1:1)] = 0.62; UV (EtOH): λ_{max} = 233, 265, 294 nm. ^1H NMR (200 MHz, $CDCl_3$): 5.75 (1H, d, J = 7.82 Hz, H-1'); 5.32 (1H, dd, J = 7.47, 5.22 Hz, H-2'); 4.33 (1H, d, J = 5.31 Hz, H-3'); 4.24 (1H, m, H-4'); 3.64 (3H, s, OCH_3); 0.96-0.87 (21H, m, TiPS).

N-Bz-5'-MMT-3'-TiPS-G: m.p. 131-133°C, R_f [$CHCl_3/Et_2O$ (1:1)] = 0.37; UV (EtOH): λ_{max} = 236, 268, 296 nm. ^1H NMR (200 MHz, $CDCl_3$): 5.70 (1H, d, J = 6.7 Hz, H-1'); 4.92 (1H, m, H-2'); 4.58 (1H, dd, J = 2.8, 5.5 Hz, H-3');

4.15 (1H, m, H-4'); 3.65 (3H, s, OCH_3); 0.99-0.88 (21H, m, TiPS).

5.3.2 5'-Monomethoxytrityl-2'-Triisopropylsilyladenosine

To a solution of 5'-MMT-A (850 mg, 1.58 mmol.) in anhydrous DMF (10 mL) were added imidazole (214 mg, 3.15 mmol.) and TiPSCl (606 mg, 3.15 mmol.). The reaction was stirred 6 hours at R.T. and was treated as in 5.3.1. The product was purified by column chromatography on silica gel (1.5 cm diameter, 15 g) using 13% Hexanes/ethyl acetate to yield 62% and 31% respectively of the 2' and 3' isomers as white foams upon removal of solvents under reduced pressure.

5'-MMT-2'-TiPS-A: R_f [13% Hexanes/ethyl acetate] = 0.57; UV (EtOH): λ_{max} = 232, 262 nm. ^1H NMR (200 MHz, $CDCl_3$): 5.85 (1H, d, J = 6.3 Hz, H-1'); 4.98 (1H, dd, J = 6.2 Hz, H-2'); 4.45 (1H, m, H-3'); 4.36 (1H, dd, J = 1.2, 4.5 Hz, H-4'); 3.74 (3H, s, OCH_3); 1.97-0.85 (21H, m, TiPS)

5'-MMT-3'-TiPS-A: R_f [13% Hexanes/ethyl acetate] = 0.32; UV (EtOH): λ_{max} = 233, 260 nm. ^1H NMR (200 MHz, $CDCl_3$): 5.93 (1H, d, J = 6.5 Hz, H-1'); 4.79 (1H, dd, J = 6.3 Hz, H-2'); 4.64 (1H, m, H-3'); 4.34 (1H, m, H-4'); 3.76 (3H, s, OCH_3); 1.01-0.88 (21H, m, TiPS).

5.3.3 Preparation of the GpC Dinucleotide (110)

All flasks were oven dried, flushed with nitrogen and equipped with septa prior to use. All solution transfers were effected via syringe. To a solution of 2,4,6-trimethylpyridine (0.17 mL, 1.24 mmol.) in anhydrous THF cooled to -78°C (isopropanol/dry ice) was added trichloroethylphosphodichloridite (TCE-PCl_2) (31 μL, 0.17 mmol.). A solution of N-Bz-5'-MMT-2'-TiPS-G (125 mg, 0.15 mmol.) in THF (1 mL + rinse (2 x 0.3 mL)) was added dropwise over a 5 minute period. After stirring 10 minutes, a solution of N-Bz-2',3'-diTBDMS-C (71.5 mg, 0.12 mmol.) in THF (1 mL + rinse (2 x 0.3 mL)) was added dropwise and the reaction was allowed to warm gradually to R.T. (30-45 minutes). A 0.1 M solution of iodine (10.2 g/134 mL H_2O/266 mL THF) was added dropwise with stirring until the brown colour remained permanently. The mixture was taken up in dichloromethane (10 mL) and washed with a 5% solution of sodium bisulfite until the mixture became colourless. The aqueous layer was extracted twice with dichloromethane (2

x 4 mL) and the combined organic layers were evaporated to dryness. The product (**109**) was purified by chromatography on two silica gel plates using EtOH/$CHCl_3$/Et_2O (3:26:71) as the eluting mixture and was isolated as a white powder in 65% yield. m.p. 138-142 °C; R_f [EtOH/$CHCl_3$/Et_2O (3:26:71)] = 0.44; UV (EtOH): λ_{max} = 234, 262, 302 nm. ^1H NMR (200 MHz, $CDCl_3$): 5.75, 5.64 (2H, 2m, H-1'); 5.39, 5.24 (2H, 2m, H-2'); 4.47, 4.39, 4.25 (4H, 3m, H-3', H-4'); 3.90, 3.68 (4H, 2m, H-5'); 3.60 (3H, s, OCH_3); 0.88 (39H, m, TiPS, t-butyl); 0.03 (12H, m, CH_3Si). ^{31}P NMR (WH-90, $CDCl_3$): -1.83, -1.11.

The dinucleotide (**109**) (500 mg, 0.32 mmol.) was detritylated by dissolving in a cooled (0°C) 0.1 M solution of benzenesulfonic acid (BSA) in acetonitrile (15 mL). The reaction was monitored by TLC [EtOH/$CHCl_3$ (1:9)]. The starting material disappeared after 40 minutes and at this time, the reaction was neutralised by addition of a saturated sodium bicarbonate solution. The acetonitrile was removed under reduced pressure and the mixture was taken up in dichloromethane (15 mL). After washing with water (2 x 4 mL), the dichloromethane was evaporated and the mixture was purified by chromatography on two silica gel plates using 5% EtOH/CH_2Cl_2. The product (**110**) was obtained in 90% yield as a white powder. m.p. 159-163 °C; R_f [10% EtOH/$CHCl_3$] = 0.56; UV (EtOH): λ_{max} = 262, 304 nm. ^1H NMR (200 MHz, $CDCl_3$): 5.75 (2h, m, H-1'); 5.27 (2H, m, H-2'); 4.66, 4.58 (4H, 2m, H-3', H-4'); 4.35, 4.10 (4H, 2m, H-5'); 0.88 (39H, m, TiPS, t-butyl); 0.07 (12H, m, CH_3Si). ^{31}P NMR (WH-90, $CDCl_3$): -1.91, -1.11.

5.4 Investigation of 2,4-Dinitrobenzenesulfenyl as a 5'-Hydroxyl Protecting Group for Use with the Phosphodichloridite Procedure : (Chapter 4)

5.4.1 β-Cyanoethylphosphodichloridite 119

The dichloridite was prepared according to procedures described in the literature [134, 140] with minor modifications. All the following manipulations were performed under a constant stream of argon. Phosphorous trichloride (BDH) (43.6 mL, 0.5 mol.), pyridine (40.4 mL, 0.5 mol.) and anhydrous diethyl ether (100 mL from a freshly opened bottle) were introduced into a three-necked flask equipped with a mechanical stirrer and were cooled to -78°C in a dry ice/acetone bath. A solution of 3-hydroxypropionitrile (Aldrich) (34.1 mL, 0.5 mol.) in diethyl ether (50 mL) was added dropwise over a 90 minute period. The reaction was allowed to reach R.T. and was stirred another 3 hours at which time the solution was passed through a fritted glass using argon pressure and the collected pyridinium hydrochloride was washed with diethyl ether (3 x 25 mL) in the same manner. The solution was heated to 26-30°C and the ether was slowly evaporated under a gentle stream of argon (1½-2 hours). Pyridinium hydrochloride was visible in the yellow liquid thus obtained and was filtered into a dried flask equipped with a magnetic stirrer using the same precautions described above. A small quantity of cesium fluoride was added to the liquid which was vacuum distilled with heating not exceeding the 105-115°C range. The product, a colourless liquid (b.p. 60-64°C, 0.007 KPa), was obtained in 48% yield (28.5 mL, ρ = 1.39 [134], 1.44 [298]). ^{1}H NMR (60 MHz, $CDCl_3$): 4.35 (2H, dt, J = 7.5, 2.0 Hz, CH_2OP); 2.78 (2H, t, J = 7.5 Hz, CH_2CN). ^{31}P NMR (XL-200, $CDCl_3$): 179.60. Physical properties and NMR characteristics of this compound and the following derivatives are collected in Tables XXII-XXVII.

5.4.2 Stability of the Dinitrobenzenesulfenyl Group

a) 2',3',5'-tri(t-Butyldimethylsilyl)uridine 121

The 2',3',5'-tri(t-Butyldimethylsilyl)uridine was prepared in 85% yield by suspending uridine 120 (1 g, 4.1 mmol.) in DMF (20 mL/mmol.) and adding imidazole (2.23 g, 32.8 mmol.) and t-butyldimethylsilyluridine

(2.47 g, 16.4 mmol.). After 4 hours stirring at R.T., the DMF was co-evaporated with toluene and the residue dissolved in dichloromethane. After washing with a 5% sodium bicarbonate solution and concentration of the organic layer to an oil, purification was effected by column chromatography on silica gel (20 g/g of product) using Hexanes to followed by MeOH/CH_2Cl_2 (0.5:9.5). ^1H NMR (200 MHz, $CDCl_3$): 8.52 (1H, m, NH); 0.92, 0.87, 0.86 (27H, 3s, t-butyl); 0.10, 0.09, 0.05, 0.04 (18H, 5s, CH_3^{Si}).

b) 2',3'-di(t-Butyldimethylsilyl)uridine **122**

A solution of trisilyluridine **121** (1.2 g, 2.19 mmol.) in nitromethane (20 mL) was treated with a solution of zinc bromide (13.1 mmol., 20 mL of [70 g $ZnBr_2$/5 mL H_2O/ 500 mL CH_3NO_2]) for 24 hours at R.T.. At this time, TLC monitoring [Et_2O/Hexanes (4:1)] revealed a 40/60 mixture of starting material and 2',3'-diTBDMSuridine **122**. The reaction was then heated to 60°C for 6 hours but was still not complete and more zinc bromide solution was added (5.3 mmol., 8 mL). Despite the continued presence of trisilyluridine, the reaction was stopped 1 hour later by addition of a 1 M ammonium acetate solution (100 mL) because of the increasing amount of base line material. The product was extracted into dichloromethane (3 x 50 mL) which was washed with a saturated sodium chloride solution (50 mL) and concentrated under reduced pressure. The oil obtained was purified by column chromatography on silica gel (2 cm diameter, 20 g) to yield 87% of a white powder. ^1H NMR (200 MHz, $CDCl_3$): 8.77 (1H, m, NH); 3.02 (1H, dd, J = 7.4, 3.0 Hz, OH-5'); 0.92-0.86 (18H, 5s, t-butyl); 0.07-0.01 (12H, 4s, CH_{3Si}).

c) 5'-Dinitrobenzenesulfenyl-2',3'-di(t-Butyldimethylsilyl)uridine **123**

To a solution of 2',3'-diTBDMSuridine (900 mg, 1.9 mmol.) in anhydrous pyridine (10 mL) was added dinitrobenzenesulfenyl chloride (581 mg, 2.50 mmol.) and the mixture was stirred at R.T. with regular TLC monitoring [Et_2O/Hexanes (4:1)]. After 5 hours, the starting material had disappeared and 2 faster moving compounds were visible, the reaction was stopped by addition of water (10 mL) and the pyridine was co-evaporated with toluene. The residue was dissolved in dichloromethane (20 mL) and washed with a 5% solution of sodium bicarbonate (10 mL) which was then extracted twice with dichloromethane. The organic portions were concentrated and purified by

repeated column chromatography on silica gel (3 cm diameter, 50 g) using first Et_2O/Hexanes (3:1) then EtOAc/Hexanes (1:3) to yield 51% of 5'-DNBS-2',3'-diTBDMSuridine **123**. ^1H NMR (200 MHz, $CDCl_3$): 9.39 (1H, m, NH); 9.13 (1H, d, J = 2.3 Hz, H-3 DNBS); 8.51 (1H, dd J = 9.1, 2.3 Hz, H-5 DNBS); 7.95 (1H, d, J = 9.0 Hz, H-6 DNBS); 0.92, 0.89 (18H, 2s, t-butyl); 0.15-0.09 (12H, 4s, CH_3Si).

d) N-Dinitrobenzenesulfenyl-2',3',5'-tri(t-Butyldimethylsilyl)uridine

N-DNBS-2',3',5'-triTBDMSuridine (**124**) was obtained in 22% yield by treatment of a solution of **121** (200 mg, 0.36 mmol.) in dry pyridine (30 mL) with dinitrobenzenesulfenylchloride (DNBSCl) (200 mg, 0.86 mmol.) at 0°C for 1 hour. The reaction was allowed to reach R.T., was stirred another 3 hours and poured over ice water (100 mL). The product did not precipitate cleanly and was extracted into dichloromethane (3 x 30 mL). The combined organic layers were concentrated and the pyridine was eliminated by co-evaporation with toluene. The oil thus obtained was purified by short column chromatography on silica gel (2 cm diameter, 10 g) with Et_2O/Hexanes (2:1). ^1H NMR (200 MHz, $CDCl_3$): 9.14 (1H, d, J =2.2 Hz, H-3 DNBS); 8.33 (1H, dd, J = 1.8, 9.1 Hz, H-5 DNBS); 8.24 (1H, d, J = 8.25, H-6); 7.11 (1H, d, J = 8.9 Hz, H-6 DNBS); 0.95, 0.88, 0.85 (27H, 3s, t-butyl); 0.14, 0.13, 0.07, 0.05 (18H, 4s, CH_3Si).

e) Stability of the DNBS Group to Condensation Conditions

To a stirring solution of 2,4,6-trimethylpyridine (0.3 mL, 2.81 mmol.) and β-cyanoethylphosphodichloridite (46 l, 0.47 mmol.) in dry THF (1 mL) cooled to 0°C, was added dropwise over a 15-20 minute period, a solution of 5'-DNBS-2',3'-diTBDMSuridine (229 mg, 0.34 mmol.) in THF (2 mL). After another 20 minutes at 0°C and 90 minutes at R.T., the starting material remained intact [TLC: MeOH/CH_2Cl_2 (3:7)]. The reaction was stopped by addition of a sodium bicarbonate solution, evaporated and extracted into dichloromethane. Following column purification on silica gel with EtOAc/Hexanes (3:1), 86% of the starting material was recovered.

f) Stability of the DNBS Group to the Oxidation Conditions

An iodine solution (1.5 mL, 0.33 mmol., 0.2 M in H_2O/THF (1:3)) was added to 5'-DNBS-2',3'-diTBDMSuridine (101 mg, 0.15 mmol.) and 2,4,6-

trimethylpyridine (0.13 mL, 1.20 mmol.) in THF (1 mL). After stirring 5 minutes at R.T., a 5% sodium bisulfite aqueous solution was added dropwise until the iodine colour no longer persisted. The mixture was extracted into dichloromethane and the starting material was recovered intact.

5.4.3 2'-Dimethoxytrityluridine **129**

a) **129** via 3',5'-di(t-Butyldimethylsilyl)-2'-Dimethoxytrityluridine **127**

The 3',5'-diTBDMSuridine **125** was prepared in 40% yield according to a method developed by Ogilvie *et al.*[236] in which 1,4-diazabicyclo[2.2.2]-octane (DABCO) (7.83 g, 69.8 mmol.) and silver nitrate (4.35 g, 25.6 mmol.) were dissolved in dry THF (20 mL/mmol.) for 10 minutes. t-Butyldimethylsilyl chloride (4.04 g, 26.8 mmol.) was added and after stirring another 10 minutes, uridine (2.84 g, 11.6 mmol.) was introduced and the reaction stirred overnight at R.T.. Silver chloride was filtered off and the product purified by repeated column chromatography on silica gel (20 g/g of product) using Et_2O/Hexanes (1:1). The product was isolated as a white foam. ^1H NMR (200 MHz, $CDCl_3$): 8.96 (1H, s, NH); 2.90 (1H, m, OH-2'); 0.91 (18H, m, t-butyl); 0.10 (12H, m, $SiCH_3$). 2',5'-diTBDMS-U (126) was isolated in 35% yield as a white foam. ^1H NMR (200 MHz, $CDCl_3$): 8.79 (1H, bs, NH); 2.65 (1H, d, J = 5.6 Hz, OH-3'); 0.91 (18H, m, t-butyl); 0.09 (12H, m, $SiCH_3$).

The disilyluridine (2.2 g, 4.65 mmol.) was dissolved in anhydrous pyridine (60 mL), dimethoxytrityl chloride (4.74 g, 13.96 mmol.) was added and the reaction stirred overnight at 70°C. Although starting material was still apparent by TLC monitoring, isomerisation of **125** to the 2',5'-disilyl was also becoming important and the reaction was stopped by addition of ethanol. The pyridine was repeatedly co-evaporated with ethanol under reduced pressure. The 3',5'-diTBDMS-2'-DMTuridine **127** was purified by column chromatography on silica gel (5 cm diameter, 130 g) with Et_2O/Hexanes (2:1) containing traces of triethylamine and was obtained in 40% yield as a yellowish foam. ^1H NMR (200 MHz, $CDCl_3$): 7.40, 6.70 (14H, 2m, Ph, H-6); 3.70, 3.67 (6H, 2s, OCH_3); 0.96, 0.78 (18H, 2s, t-butyl); 0.00 (12H, m, $SiCH_3$).

The silyl groups were removed by dissolving **127** (850 mg, 1.09 mmol.) in dry THF (4 mL) and adding tetrabutylammonium fluoride (TBAF) (5.45 mmol., 10.5 mL, 1M solution in THF, Aldrich). After stirring overnight at R.T., H_2O was added and the THF was removed under reduced pressure. The product was extracted into dichloromethane and washed with a 5% solution of sodium bicarbonate before being concentrated to an oil. This was purified by column chromatography on silica gel (2 cm diameter, 17 g) with distilled ethyl acetate containing a few drops of triethylamine. 2'-DMT-U **129** was obtained in 74% yield as a white foam. ^1H NMR (300 MHz, $CDCl_3$): 7.31, 6.76 (14H, 2m, Ph, H-6); 3.74, 3.72 (6H, 2s, OCH_3). ^1H NMR (200 MHz, dmso-d6): 11.26 (1H, m, NH); 7.45, 6.75 (14H, 2m, Ph, H-6); 5.02 (1H, t, OH-5'); 4.96 (1H, d, J = 4.9 Hz, OH-3');

b) **129** via 5'-t-Butyldimethylsilyl-2'-Dimethoxytrityluridine **132**

5'-TBDMSuridine **131** was prepared in 83% yield by the method described by Ogilvie *et al.*[122]. Uridine (5 g, 20.5 mmol.) was first dissolved in dry DMF (40 mL); imidazole (3.6 g, 53.3 mmol.) and t-butyldimethylsilyl chloride (4.0 g, 26.7 mmol.) were added and the solution stirred 3 hours at R.T.. The consumption of starting material was confirmed by TLC [MeOH/CH_2Cl_2 (0.5:9.5)] and the reaction was stopped by addition of a 5% sodium bicarbonate solution (15 mL). After co-evaporation of DMF with ethanol, the residue was dissolved in dichloromethane (50 mL) and washed with a 5% sodium bicarbonate solution. The product was purified to a white foam by column chromatography on silica gel (5 cm diameter, 150 g) using EtOH/CH_2Cl_2 (0.5:9.5). ^1H NMR (200 MHz, $CDCl_3$): 2.94, 2.86 (2H, 2m, OH-2', OH-3'); 0,90 (9H, s, t-butyl); 0.09 (6H, s, $SiCH_3$).

5'-TBDMS-U (4.1 g, 11.3 mmol.) was thoroughly dried under reduced pressure then dissolved in dry pyridine (40 mL). Dimethoxytrityl chloride (5.74 g, 16.95 mmol.) was added and the solution was stirred 14 hours at R.T.. The disappearance of starting material was monitored by TLC and, if necessary, dimethoxytrityl chloride (1.15 g, 3.40 mmol.) was again added and the reaction allowed to proceed for 3 hours more. Excess trityl chloride was destroyed by addition of water (15 mL) and pyridine was eliminated by co-evaporation with toluene under reduced pressure. The residue was taken up in dichloromethane (40 mL), washed with a 5% sodium bicarbo-

nate solution (20 mL) to eliminate any pyridinium hydrochloride, and concentrated to an oil. This was purified by column chromatography on silica gel (6 cm diameter, 180 g) using Et_2O/CH_2Cl_2 (1:3) to yield 76% of 5'-TBDMS-2'-DMTuridine **132** as a white foam. ^1H NMR (200 MHz, $CDCl_3$): 7.28, 6.84 (13H, m, Ph); 3.79 (6H, 2s, OCH_3); 0.91 (9H, s, t-butyl); 0.11 (6H, s, $SiCH_3$). 5'-TBDMS-3'-DMT-U **133** was obtained in 22% yield. ^1H NMR (200 MHz, $CDCl_3$): 7.28, 6.85 (13H, 2m, Ph); 3.80 (6H, 2s, OCH_3); 0.78 (9H, s, t-butyl); 0.00, -0.05 (6H, 2s, $SiCH_3$).

2'-DMT-U was obtained in 88% yield by treating a solution of 5'-TBDMS-2'DMT-U **132** (1 eq.) in dry THF (4 mL/mmol.) with TBAF (1.5 eq., 1 M in THF) at R.T. for 2 hours. The reaction was monitored by TLC [EtOAc]; work-up and purification were effected as in 5.4.1a (20 g of silica gel/g of product).

c) **129** via 3',5'-tetraisopropyldisiloxyl-2'-dimethoxytrityluridine **136**

Uridine (244 mg, 1 mmol.) was dissolved in anhydrous pyridine (20 mL) under nitrogen atmosphere. The flask was fitted with a septum and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TiPDSCl_2$) (0.35 g, 1.1 mmol.) was added via syringe. The reaction was stirred at R.T. and monitored by TLC [$MeOH/CH_2Cl_2$ (3:7)]. After one hour, water (5 mL) was added and the pyridine was co-evaporated with toluene under reduced pressure. Attempts to crystallise the resulting oil were not successful and the product was purified by column chromatography on silica gel (2 cm diameter, 10 g) using Et_2O/CH_2Cl_2 (1:2) to yield 70% of 3'5'-TiPDSuridine (**134**) as a white solid. ^1H NMR (200 MHz, $CDCl_3$): 1.05 (28H, TiPDS). The 2',3'-TiPDSuridine derivative **135** was isolated in 8% yield. ^1H NMR (200 MHz, $CDCl_3$): 1.03 (28H, m, TiPDS).

To a suspension of 3',5'-TiPDSuridine **134** (200 mg, 0.41 mmol.) in dry THF (20 mL) were added pyridine (0.22 mL, 2.65 mmol.), silver nitrate (91 mg, 0.53 mmol.) and dimethoxytrityl chloride (180 mg, 0.53 mmol,). The reaction was heated at 60°C overnight and monitored by TLC [Et_2O/CH_2Cl_2 (1:3)]. If starting material was still apparent, additional silver nitrate (0.21 mmol.), pyridine (1.03 mmol.) and dimethoxytrityl chloride (0.21 mmol.) were introduced and the reaction heated for another 3 hours. The mixture was filtered to eliminate the silver chloride formed and

evaporated under reduced pressure. The residue was taken up in dichloromethane, washed with a 5% sodium bicarbonate solution and concentrated to an oil which was purified by column chromatography on silica gel (2 cm diameter, 15 g) using Et_2O/CH_2Cl_2 (1:3) to yield 97% of 3',5'-TiPDS-2'-DMTuridine (**136**). ^1H NMR (200 MHz, $CDCl_3$): 7.78 (1H, m, NH); 7.47, 6.72 (13H, 2m, Ph); 3.75 (6H, 2s, OCH_3); 1.07 (28H, m, TiPDS).

The preceding compound (**136**) (1 eq.) was dissolved in anhydrous THF (4 mL/mmol.) and treated with TBAF (4 eq., 1 M in THF) at room temperature for 2 hours. The reaction was monitored by TLC [EtOAc] then worked up and purified as in 5.4.1b to yield 98% of 2'-DMTuridine **129**.

5.4.4 5'-Dinitrobenzenesulfenyl-2'-Dimethoxytrityluridine **137**

2'-DMTuridine **129** (3.35 g, 6.1 mmol.) was dissolved in dry pyridine (20 mL) under argon atmosphere in a flask equipped with a septum. The solution was cooled to 0°C and a solution of dinitrobenzenesulfenyl chloride (2.16 g, 9.2 mmol.) in dry pyridine (15 mL) was added dropwise via syringe over a 20 minute period. The reaction was stirred 1 hour at 0°C then 14 hours a R.T.. Monitoring by TLC [Et_2O/CH_2Cl_2 (1:1)] revealed the presence of starting material and desired product as well as a small quantities of faster moving sulfenylated material. The mixture was poured into ice water (400 mL) which was allowed to stand until completely melted. The product was collected by filtration, washed abundantly with water and dried thoroughly. The solid (5.32 g) was taken up in dichloromethane (20 mL) and filtered to eliminate the disulfide formed. The oil obtained upon evaporation was purified by repeated column chromatography on silica gel (5 cm diameter, 130 g) using Et_2O/CH_2Cl_2 (1:3). The fast moving sulfenylated material (459 mg), a mixture of disulfenylated products, accounted for 8% of recovered yield whereas the 3'-sulfenylated isomer **138** (816 mg) was obtained in 18% yield. When the reaction was performed on 2'-DMT-U containing traces of 3'-DMT-U, the 5'-DNBS-3'DMT derivative was also formed but this could not be separated from **138**. The desired 5'-DNBS-2'-DMTuridine (**137**) was isolated in 47% yield (2.13 g). ^1H NMR (200 MHz, $CDCl_3$): 9.53 (1H,m, NH); 9.10 (1H, d, J = 2.3 Hz, H-3 DNBS); 8.48 (1H, dd, J = 9.1, 2.3 Hz, H-5 DNBS); 7.72 (1H, d, J = 9.1 Hz, H-6 DNBS); 7.30, 6.82 (13H, 2m, Ph); 3.48, 3.45 (6H, 2s, OCH_3); 2.48 (1H, m, OH-3').

3'-DNBS-2'DMT-U **138**: ^1H NMR (200 MHz, $CDCl_3$): 9.27 (1H, m, NH); 9.08 (1H, d, J = 2.0 Hz, H-3 DNBS); 8.45 (1H, dd, J = 9.1, 2.0 Hz, H-5 DNBS); 7.72 (1H, d, J = 9.1 Hz, H-6 DNBS); 3.78 (8H, m, H-5', OCH_3); 3.07 (1H, dd, J = 4.7 Hz, H-3').

5'-DNBS-3'-DMT-U **139**: ^1H NMR (200 MHz, $CDCl_3$): 9.05 (1H, d, J = 2.2 Hz, H-3 DNBS); 8.62 (1H, m, NH); 8.22 (1H, dd, J = 9.1, 2.4 Hz, H-5 DNBS); 7.96 (1H, d, J = 9.1 Hz, H-6 DNBS); 7.25, 6.79 (14H, 2m, H-6, Ph); 3.75 (8H, m, H-5', OCH_3); 3.00 (1H, m, OH-2').

5.4.5 Removal of the Dinitrobenzenesulfenyl Group

a) A freshly prepared solution of triethylammonium thiocresolate (360 mg thiocresol/0.42 mL TEA/4.6 mL THF) was added to 5'-DNBS-2'-DMTuridine **137** (50 mg, 0.07 mmol.) in dry THF (1.5 mL) causing the solution to turn brown instantly. The reaction was stirred at R.T. and monitored at 5 minute intervals [TLC: Et_2O/CH_2Cl_2 (1:1)] until only 2'-DMTuridine was present. After 20 minutes, ethanol was added and the solvents were evaporated under reduced pressure without allowing the temperature to rise above 35°C. When the thiocresol odour persisted, toluene was added and evaporated as above the product (**129**) was purified on a silica gel plate using ethyl acetate containing traces of triethylamine and recovered in 60% yield.

b) The reaction was repeated as described above using a solution of thiocresol (0.4 mL, 0.20 mmol., 0.5 M in dry THF). TLC monitoring revealed considerable detritylation of the product after 20 minutes.

5.4.6 Stability of the β-Cyanoethyl Group to the conditions for removal of the DNBS group

To a solution of the 3'-bis(cyanoethyl)phosphotriester of N-benzoyl-5'-monomethoxytrityl-2'-t-butyldimethylsilyladenosine **140** (47 mg, 0.05 mmol.) in dry THF (0.5 mL) was added a solution of thiocresol (0.15 mmol., 0.3 mL, solution 5.4.6b). The mixture was stirred at R.T. and monitored by TLC [$MeOH/CH_2Cl_2$ (3:7)]. The compound was stable for 30 minutes (R_f = 0.49). After 45 minutes, a slower moving tritylated spot appeared (R_f = 0.14) and became the most important component when the reaction was left overnight.

5.4.7 Condensation Procedure

All glassware used was oven dried, flushed with argon and equipped with a septum. All transfers were effected via syringe. To a cooled solution (0°C) of 2,4,6-trimethylpyridine (0.92 mL, 6.93 mmol.) in dry THF was added β-cyanoethylphosphodichloridite (143 μL, 1.16 mmol.). A solution of 5'-DNBS-2'-DMTuridine **137** (781 mg, 1.05 mmol.) in THF (4 mL + rinse (2 x 0.5 mL)) was added dropwise over a 20 minute period. The reaction was stirred another 20 minutes at 0°C and a solution of 2'-DMTuridine **129** (460 mg, 0.84 mmol.) in THF (2 mL + rinse (2 x 0.5 mL)) was added dropwise over 15 minutes. The reaction was allowed to reach R.T. and stirred for 1½ hours. A 0.1 M solution of iodine (10.2 g/134 mL H_2O/266 mL THF) was added dropwise with vigorous stirring until the brown colour persisted and the reaction was stirred 1 minute more. The mixture was then transferred (with chloroform rinsing) into a 250 mL separatory funnel containing chloroform (60 mL), H_2O (20 mL) and a 5% sodium bisulfite solution (3 mL) and shaken until disappearance of the iodine colour (1-2 minutes). After repeated chloroform extractions (3 x 40 mL), washing of the combined extracts with a 5% sodium bicarbonate solution (40 mL) and solvent evaporation, a yellow foam was obtained. This was purified by column chromatography on silica gel (2 cm column, 50 g) with $CHCl_3$/EtOAc (1:9) containing traces of triethylamine and the collected fractions were precipitated from distilled hexanes to eliminate residual 2,4,6-collidine. The 2 diastereomers of the desired 3',5'-dimer **141** were obtained as a yellowish powder in 50% yield. ^{1}H NMR (200 MHz, $CDCl_3$): 9.58, 9.45 (2m, NH); 9.08 (d, J = 2.2 Hz, H-3 DNBS); 8.46 (m, H-5 DNBS); 8.39 (dd, J = 2.2, 9.0 Hz, H-5 DNBS); 7.66 (d, J = 9.2 Hz, H-6 DNBS); 7.41 (m, H-6 DNBS); 7.27, 6.70 (2m, Ph, H-6, H-6); 4.57 (m, H-2', CH_2OP); 3.73, 3.72 (2m, OCH_3, H-3'); 2.66 (m, H-3', CH_2CN). ^{31}P NMR (XL-200, $CDCl_3$): -2.37; -2.71.

The 3',3'-dimer **142** was isolated in 8% yield. ^{1}H NMR (200 MHz, $CDCl_3$): 9.54 (1H, m, NH); 9.39 (1H, m, NH); 9.04 (1H, d, J = 1.6 Hz, H-3 DNBS); 8.98 (1H, d, J = 2.2 Hz, H-3 DNBS); 8.26 (1H, dd, J = 2.0, 9.9 Hz, H-5 DNBS); 8.09 (1H, dd, J = 1.7, 9.0 Hz, H-5 DNBS); 7.54 (1H, d, J = 9.7 Hz, H-6 DNBS); 7.22, 6.76 (28H, 2m, Ph, H-6 DNBS, H-6); 4.30 (2H, m, CH_2OP); 3.76, 3.74, 3.70 (12H, 3s, OCH_3); 2.50 (2H, t, CH_2CN). ^{31}P NMR (XL-200,

$CDCl_3$): -2.51.

5.4.8 Deprotection of Dinucleotide **141**

a) Removal of the Dinitrobenzenesulfenyl Group

All glassware was oven dried, flushed with argon and equipped with a septum. To a solution of the dinucleotide (185 mg, 0.13 mmol.) in anhydrous THF (2 mL) was added, via syringe, a solution of thiocresol in triethylamine/THF (0.39 mmol., 0.78 mL, solution 5.4.6b). The mixture turned to a brown colour immediately and was stirred at R.T. with constant TLC monitoring [EtOH/CH_2Cl_2/EtOAc (3:10:87)]. The reaction was complete after 25 minutes and some detritylation was apparent. After work-up as in 5.4.6a, the product was purified by column chromatography on silica gel (3 cm diameter, 15 g) using CH_2Cl_2/EtOAc (1:9) containing traces of triethylamine to yield 45% of dinucleotide **143**. ^{1}H NMR (200 MHz, $CDCl_3$): 7.31, 6.74 (28H, 2m, Ph, H-6, H-6); 4.43 (2H, m, CH_2OP); 3.71 (12H, m, OCH_3); 2.70 (2H, m, CH_2CN).

b) Removal of the β-Cyanoethyl and Dimethoxytrityl Groups

Dinucleotide **143** (35 mg, 0.03 mmol.) was treated with 40% triethylamine in acetonitrile (3 mL). The reaction turned purple instantly and was stirred overnight at R.T.. The solvents were removed under reduced pressure and residual triethylamine was eliminated by co-evaporation with toluene. A TLC [EtOAc] taken at this point showed only tritylated base line material as expected for complete removal of the β-cyanoethyl group.

From this point in the procedure, sterile handling techniques were used for all manipulations: glassware and Pasteur pipettes were silanised by treatment with Sigmacote (Sigma) and autoclaved. Teflon test tubes were also autoclaved. Gloves were worn at all times.

The crude phosphodiester dinucleotide was dissolved in ethanol and transferred into a small teflon test tube. The solvent was removed under reduced pressure with mild heating in a speed-vac. 80% Acetic acid (0.75 mL, 10 mmol.) was added to the solution which turned orange instantly. After stirring at R.T. for 1½ hours, the mixture was cooled, neutralised to pH 6 by dropwise addition of ammonium hydroxide, frozen in dry ice and evaporated overnight in a speed-vac. The material obtained revealed one

spot only by TLC on cellulose and was estimated at 290 O.D. units by UV absorption at 260 nm. The combined yield for the removal of the -cyanoethyl and dimethoxytrityl groups was 54% (0.016 mmol.). A few drops of autoclaved H_2O were added and the residue was purified on a cellulose coated plate using isoPrOH/NH_4OH/H_2O (63:10:27). The cellulose band was scraped off and suspended in 5 mL of autoclaved H_2O in a teflon tube. The mixture was stirred on an automatic vortex then centrifuged and the supernatant was gently collected. The elution steps were repeated 4 times and the combined supernatants were concentrated in a speed-vac. The completely deprotected dinucleotide **144** was obtained in 23% yield (122 O.D. units) following the purification (42% yield of recovery from cellulose plate).

c) Confirmation of Structure by Enzyme Degradation

To a solution of the deprotected dinucleotide **144** (0.2 O.D. units) in 0.5 M ammonium acetate buffer (75 l; adjusted to pH 6.5 with acetic acid) was added a solution of spleen phosphodiesterase (10 l, 2 mg/mL in ammonium sulfate buffer; Boehringer Mannheim). After 30 minutes incubation at 37°C, the composition was determined by isocratic elution HPLC analysis with 1% NH_4OAc/H_2O, pH 5.9) [294]. Uridine and the 3'-uridine phosphate monoester were observed in a 1:1 ratio.

5.4.9 Reactions of Zinc Bromide with Dimethoxytritylated Uridine

a) Preparation of 2',5'-Di(Dimethoxytrityl)uridine **145**

2',5'-diDMTuridine was prepared by a procedure developed in our laboratory [236] with minor modifications: uridine (2 g, 8.19 mmol.) was first suspended in THF (14 mL/mmol.); pyridine (6.6 mL, 81.9 mmol.), silver nitrate (2.78 g, 16.38 mmol.) and dimethoxytrityl chloride (5.55 g, 16.38 mmol.) were then added. After overnight stirring, the reaction was filtered into a 5% sodium bicarbonate solution, extracted into dichloromethane and concentrated to an oil which was purified by repeated short column chromatography on silica gel (20 g/g of material) using Et_2O/CH_2Cl_2 (1:2) containing a few drops of triethylamine. The 2',5' and 3',5'-isomers were isolated in yields of 50 and 24% respectively (reported yields: 80% and 15%). 2',5'-diDMTuridine **145**: ^1H NMR (200 MHz, $CDCl_3$): 9.99 (1H, s, NH); 7.47, 6.67 (26H, 2m, Ph); 3.78, 3.77, 3.76, 3.73 (12H, 4s, OCH_3). ^1H NMR

(200 MHz, dmso-d6): 11.22 (1H, m, NH); 7.36, 6.72 (27H, 2m, Ph, H-6); 6.08 (1H, d, J = 7.1 Hz, H-1'); 5.14 (1H, d, J = 8.2 Hz, H-5); 4.77 (1H, d, J = 5.4 Hz, OH-3'); 4.25 (1H, m, H-2'); 3.87 (1H, bs, H-4'); 3.71 (7H, bs, OCH_3, H-3'); 2.95 (2H, bs, H-5'). 3',5'-diDMT-U **146**: m.p. 136-137°C; R_f [Et_2O/CH_2Cl_2 (1:1)] = 0.47; UV (EtOH): λ_{max} = 265, 233 nm. ^1H NMR (200 MHz, $CDCl_3$): 8.77 (1H, m, NH); 7.22, 6.75 (26H, 2m, Ph); 3.78, 3.76, 3.75 (12H, 3s, OCH_3). ^1H NMR (200 MHz, dmso-d6): 11.4 (1H; m, NH); 8.57, 7.22 (27H, 2m, Ph, H-6); 6.02 (1H, d, J = 7.0 Hz, H-1'); 5.89 (1H, d, J = 6.5 Hz, OH-2'); 5.41 (1H, d, J = 8.0 Hz, H-5); 4.05 (1H, m, H-2'); 3.98 (1H, m, H-4'); 3.68, 3.67 (6H, 2s, OCH_3); 3.09 (1H, m, H-3'); 2.78 (1H, d, J = 9.6 Hz, H-5'); 2.55 (1H, d, J = 9.2 Hz, H-5').

b) Treatment of 2',5'-Di(Dimethoxytrityl)uridine with Zinc Bromide

2',5'-diDMTuridine **145** (3 g, 3.53 mmol.) was dissolved in anhydrous nitromethane under argon atmosphere. The stirred solution was cooled to 0-5°C and treated with zinc bromide (17.7 mmol., 177 mL of a 0.1 M solution in dry CH_3NO_2). The solution became orange instantly and monitoring was effected at 5 minute intervals by TLC [EtOAc]. No traces of starting material were present after 30 minutes and the reaction was quenched by addition of ammonium acetate (1 M solution in H_2O) until disappearance of the orange colour. The solution was extracted into dichloromethane and concentrated to an oil which was purified by column chromatography on silica gel (20 g/g of product) with distilled ethyl acetate containing a few drops of triethylamine. 5'-DMTuridine **147** was obtained as a white foam in 91% yield. ^1H NMR (200 MHz, dmso-d6): 11.37 (1H, m, NH); 7.39, 6.87 (13H, 2m, Ph); 5.50, 5.16 (2H, 2d, J = 4.8, 5.6 Hz, OH-2', OH-3'); 3.73 (6H, s, OCH_3).

c) Treatment of 5'-Dimethoxytrityluridine **147** with Zinc-Bromide

With the same precautions as in 5.4.9b, the 5'-DMTuridine (200 mg, 0.37 mmol.) was dissolved in dry nitromethane (15 mL) and treated with zinc bromide (4.44 mmol., 44.4 mL, 0.1 M in dry CH_3NO_2) at 0-5°C. Monitoring by TLC [EtOAc and MeOH/CH_2Cl_2 (3:7)] showed the disappearance of starting material after 10 minutes and the presence of a slower moving compound. After quenching with ammonium acetate, the solution was directly concentrated under reduced pressure. The residue was dissolved in water

and washed with diethyl ether to eliminate the tritanol. Following evaporation, the product was identified as uridine by ultraviolet absorption. UV (EtOH): λ_{max} = 260 nm; λ_{min} = 232 nm.

d) Reaction of 3',5'-Di(Dimethoxytrityl)thymidine 148 with Zinc Bromide

A solution of 3',5'-diDMTthymidine (200 mg, 0.24 mmol.) in dry nitromethane (10 mL) was treated with zinc bromide (2.4 mmol.; 24 mL, 1 M in dry CH_3NO_2) at 0-5°C and monitored by TLC [MeOH/CH_2Cl_2 (3:7)]. After 20 minutes, work-up was effected as in 5.4.9a. Purification of the residue by column chromatography on silica gel (2 cm diameter, 10 g) with MeOH/CH_2Cl_2 (3:7) yielded 90% of 3'-DMTthymidine 149. 3',5'-diDMT-T 148: ^{1}H · NMR (200MHz, dmso-d6): 11.32 (1H, m, NH); 7.33, 6.76 (27H, 2m, Ph, H-6); 3.71, 3.70, 3.69 (12H, 3s, OCH_3); 1.36 (3H, s, CH_3). 3'-DMT-T XX: R_f [MeOH/CH_2Cl_2 (3:7)] = 0.15. ^{1}H NMR (200 MHz, dmso-d6): 11.28 (1H, m, NH); 7.61, 6.87 (14H, 2m, Ph, H-6); 6.21 (1H, t, J = 2.05 Hz, H-1'); 4.96 (1H, m, OH-5'); 4.26 (1H, m, H-3'); 3.78 (1H, m, H-4'); 3.71 (6H, s, OCH_3); 3.36, 3.31 (2H, 2m, H-5'); 1.70 (4H, m, H-2', CH_3); 1.52 (1H, m, H-2').

e) Reaction of 2',3',5'-Tri(Dimethoxytrityl)uridine with Zinc Bromide

The product (150) was prepared in 87% yield by suspending uridine (400 mg, 1.6 mmol.) in anhydrous THF (40 mL) and successively adding dry pyridine (4 mL, 48.0 mmol.), silver nitrate (1.67 g, 9.6 mmol.) and dimethoxytrityl chloride (3.33 g, 9.6 mmol.). The reaction was heated for 6 hours at 60°C after which time it was cooled and the silver chloride filtered off and washed with dichloromethane. A 5% solution of sodium bicarbonate (20 mL) was added to the filtrate and the product was extracted into dichloromethane (3 x 20 mL). The organic fractions were combined and concentrated under reduced pressure and the pyridine was eliminated by co-evaporation with toluene. The oil thus obtained was purified by column chromatography on silica gel (3 cm diameter, 40 g) with Et_2O/CH_2Cl_2 (1:4) containing a few drops of triethylamine to yield the tridimethoxytrityluridine as a yellow foam. ^{1}H NMR (200 MHz, $CDCl_3$): 7.59 (1H, d, J = 8.3 Hz, H-6); 7.49, 6.52 (39H, 2m, Ph, H-1'); 3.78-3.62 (18H, 5s, OCH_3);

2',3',5'-Tri(Dimethoxytrityl)uridine (758 mg, 0.66 mmol.) was dissolved in anhydrous nitromethane (30 mL) and treated with zinc bromide (19.8 mmol., 198 mL, 1 M in dry CH_3NO_2) at 40°C. After 2 hours, a consid-

erable amount of starting material was still apparent by TLC [Et_2O/CH_2Cl_2 (1:1)] and more zinc bromide was added (6.6 mmol.). This reagent (6.6 mmol.) was added once more 1½ hours later as starting material was still present. The reaction was complete after a total of 4 hours showing a single product by TLC. Activated silica gel (3 g) was added and the mixture stirred for 10 minutes at R.T.. After pouring into aqueous ammonium acetate (60 mL of a 1 M solution), the product was extracted into dichloromethane (3 x 40 mL). The combined organic layers were concentrated to an oil which was spotted on TLC to reveal starting material as well as a slower migrating product. These were purified by column chromatography on silica gel (2 cm diameter, 25 g) with Et_2O/CH_2Cl_2 (1:4) containing a few drops of triethylamine to yield 62% of 2',3',5'-triDMTuridine and 19% of 2',3'-diDMTuridine **151**. ^1H NMR (200 MHz, $CDCl_3$): 7.43 (1H, m, NH); 7.20, 6.68 (26H, 2m, Ph); 3.75, 3.74, 3.71 (12H, 3s, OCH_3).

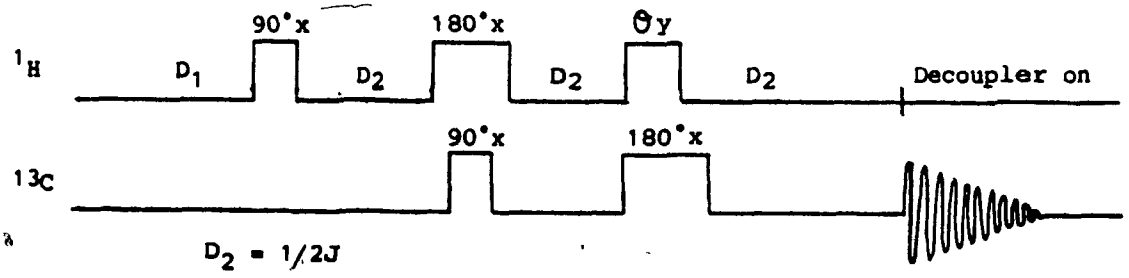
Parameters for DEPT, INEPT and HETCOR NMR Experiments

I- Parameters for ^{13}C DEPT NMR with ADEPT Editing

The distortionless enhancement by polarisation transfer (DEPT) experiment was introduced by Doddrell et al.[299, 300]. This multipulse sequence allows signal enhancement by transferring nuclear spin polarisation from the nucleus with a large Boltzman population difference, usually hydrogen, to the species under investigation, in this case carbon. Although the signal enhancement obtained by this pulse sequence is dependant on the choice of the C-H coupling constant ($J_{C,H}$), the choice is less critical in the DEPT experiment than in the INEPT (described in section II). This polarisation transfer method can also be used for the assignment of resonance signals [301]. The CH_3, CH_2 and CH possess different magnetisation phase properties. It is thus possible to generate subspectra differentiating these signals. ADEPT editing produces four subspectra in which the carbon signals are sorted according to their multiplicities. There are no protons from which polarisation can be transferred to quatenary carbons and these do not produce signals by the DEPT technique.

The DEPT pulse sequence used is shown in scheme XLIV. The final proton pulse [H,θy] reorients proton magnetisation so that all CH signals are positive (θ = 45°); only CH signals appear (θ = 90°); CH_2 peaks are negative and CH_3 peaks positive (θ = 135°).

Scheme XLIV

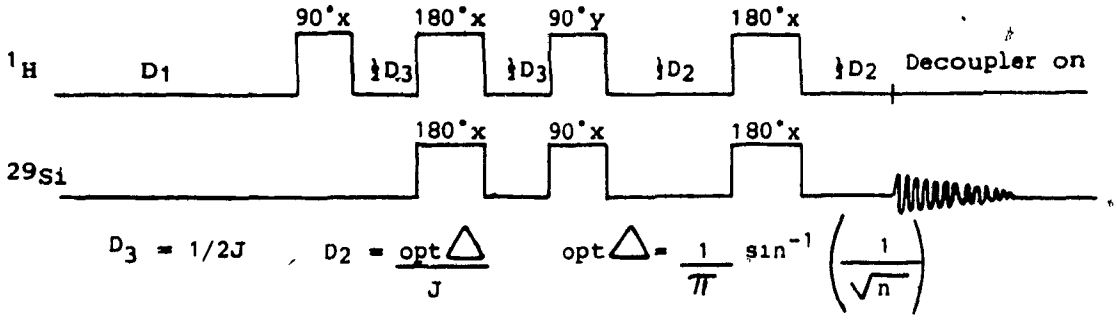


The D_1 was set at 2.0 s throughout our experiments and the C-H coupling constant (J) was set at 140 Hz ($D_2 = 3.6 \times 10^{-2}$ s).

II- Optimisation of Parameters for ^{29}Si INEPT NMR

The Insensitive Nuclei Enhancement by Polarisation Transfer (INEPT) sequence as originally described by Morris and Freeman [257] comprises an equilibration period followed by polarisation transfer. The adding of a refocusing period preceding acquisition allows for decoupled signals. The sequence was further improved by an additional refocusing proton pulse introduced by Thomas et al.[302], to reduce or eliminate distortions inherent to the original pulse sequence. The sequence used in our experiments is schematised in diagram XLV. The equilibration period is determined by the delay time D_1. The polarisation transfer is effected by a 90°[H,x] pulse and, after a free precessing delay D_3, simultaneous 90°[H,y] and 90°[Si,x] pulses reorient proton and silicon magnetisations. At the half point of this delay, refocusing pulses 180°[H,x] and 180°[Si,x] are applied. The refocusing period is characterised by the D_2 delay with another series of refocusing pulses at midpoint.

Scheme XLV



$D_3 = 1/2J$ $\quad D_2 = \frac{\text{opt}\Delta}{J}$ $\quad \text{opt}\Delta = \frac{1}{\pi} \sin^{-1}\left(\frac{1}{\sqrt{n}}\right)$

where n = number of hydrogen atoms coupled to silicon

J = Si-H coupling constant (Hz)

D_1 was set at 2.0 s throughout our experiments. To approximate the conditions of optimal ^{29}Si signal enhancement for TBDMS protected nucleosides, t-butyldimethylsilyl chloride in $CDCl_3$ was used as sample. Optimisation of delay times was attained by the following method.

Optimisation of D_2: $^2J_{Si-CH}$ was first arbitrarily assumed to be 6.6 Hz (value reported for the corresponding coupling in TMS [266]) and D_3 was calculated according to the equation (see above) derived by Pegg et al.[303] (7.46×10^{-2} s). A series of absolute intensity experiments were then conducted in which D_2 was varied by using different n values while maintaining $J_{Si,H}$ constant (6.6 Hz).

e.g.	n (H)	6	9	15
	D_2 (s)	0.0199	0.0160	0.0124

Optimisation of D_3: the number of coupled hydrogen atoms was set at the value for which a maximum was observed in the previous experiment (n = 9, opt = 0.1082, D_2 = 0.016 s). Another series of experiments was conducted during which D_3 was varied, using different $J_{Si,H}$ values (5 to 13 Hz). The J dependent variations of D_2 were temporarily disregarded. The absolute intensity maximum was observed for $J_{Si,H}$ = 6.5 Hz.

e.g.	$J_{Si,H}$ (Hz)	6.5	6.6	6.7	6.8	6.9
	D_3 (s)	0.0769	0.0758	0.0746	0.0736	0.0725

The conditions for recording the TiPS protected nucleosides were determined in a similar manner using triisopropylsilyl chloride. Appropriate n values were chosen (i.e. n = 3, 18, 21H) and a coupling constant range of 5 to 13 Hz was studied.

TABLE XXIX

Parameters for ^{29}Si INEPT NMR of Silylated Nucleosides

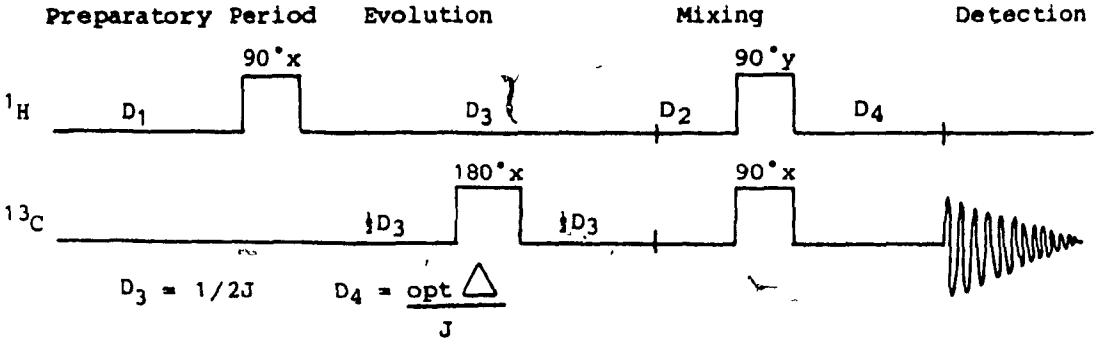
Group	n	opt Δ ($\times 10^2$)	$J_{Si,H}$ (Hz)	D_1 (s)	D_2 ($\times 10^3$ s)	D_3 ($\times 10^3$ s)
TBDMS	9	10.82	6.5	2.0	16.7	76.9
TiPS	21	7.0	8.9	2.0	7.9	56.2

The optimal parameters, summarised in Table XXIX, gave excellent signal to noise ratios for 15 to 30 mg/mL samples in 10 mm tubes after only 40 to 80 transients (1.5-3 minutes) on an XL-200 spectrometer.

III- Parameters for ^{13}C-^{1}H Hetero-correlated NMR

This multipulse sequence introduced by Maudsley, Muller and Ernst [177], permits the signal assignment of one type of nucleus, in this case carbon, from the known assignment of another (usually proton). A two dimensional matrix is generated in which the ^{1}H spectrum occupies the x axis and the ^{13}C signals, the y axis. The pulse sequence also comprises a polarisation transfer which is helpful in enhancing the carbon signals and a mixing period during which information is transferred between protons and carbon.

Scheme XLVI



The sequence used in our studies is represented in Scheme XLVI. As in the INEPT experiment, described in section II, an equilibration period, D_1, is followed by a polarisation transfer 90°[H,x] pulse. The evolution delay, D_2, is halved by a 180°[C,x] pulse which exchanges the proton spin states resulting in refocusing. The mixing period consists of delay D_3 followed by simultaneous 90°[H,y] and 90°[C,x] pulses which, as in the INEPT sequence, reorient proton and silicon magnetisations. The parameters reported in Table XXX were used in all ^{1}H-^{13}C heterocorrelated spectra.

TABLE XXX

Parameters for ^{13}C-^{1}H HETCOR NMR

$J_{C,H}$ (Hz)	D_1 (s)	D_2 (s)	D_3 (s x 10^3)	D_4 (s)
160.0	2.0	0.0	6.3	0.5

IV- Parameters for $^{29}Si-^{1}H$ Hetero-Correlated NMR

The HETCOR pulse sequence used in this experiment is the same as that described for $^{13}C-^{1}H$ spectra in section III with silicon substituted for carbon. The D_1 was set at 2.0 s and the D_2 at 0.0 s throughout our experiments. The D_3 and D_4 delays (corresponding to D_3 and D_2 in the INEPT sequence) were optimised for correlation with the corresponding sugar ring proton using 3',5'-diTBDMSuridine in $CDCl_3$ (5 mm tube).

Optimisation of D_3: the number of coupled hydrogens was arbitrarily set at 9, the value for maximal INEPT signals (Section II) and the coupling constant was set at 3.0 Hz (value reported for $^{3}J_{Si-H}$ in Si-O-C-H [275]). D_4 was maintained unchanged (opt = 0.0769, D_4 = 0.0166 s) and a series of experiments were conducted in which various $J_{Si,H}$ values (2.0 to 6.0 Hz) were used in calculating the D_3 parameter.

Optimisation of D_4: D_3 was set at the value for which correlation with the sugar protons was observed in the previous experiment (D_3 = 3.0 Hz) and various D_4 values were investigated (1 to 4 x 10^{-3} s).

TABLE XXI

Parameters for $^{29}Si-^{1}H$ HETCOR NMR of Disilylated Nucleosides

J (Hz)	D_1 (s)	D_2 (s)	D_3 (s)	D_4 (s x 10^2)
3.0	2.0	0	0.167	2.0

The best results were obtained with the parameters summarised in Table XXI and these were used for all $^{29}Si-^{1}H$ HETCOR spectra reported. The samples studied were fairly concentrated (20-30 mg/mL) and required overnight acquisition (128 x (32 to 70) transients) on an XL-300 spectrometer.

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