THE ATTEMPTED SYNTHESIS OF 2'-[2-AMINO-3(p-METHOXYPHENYL) PROPANAMIDO]-2'-DEOXY-N^6N^6-DIMETHYLADENOSINE, AN ISOMER OF THE ANTIBIOTIC AND ANTITUMOUR DRUG PUROMYCIN.

A Thesis submitted for the Degree

of

DOCTOR OF PHILOSOPHY

in the

Department of Chemistry

of

City University

by

MAHAMED ALLY SOODIN

JULY 1994

CONTENTS.

TO MY LOVING FATHER

MOUSTAPHA SOODIN

(IN VERY PAINFUL MEMORY)

(HE WENT IN SEARCH FOR HIS FAMILY'S LIVING AND LOST HIS OWN LIFE
VANISHED AT SEA FOR EVER. HE NEVER SAID GOODBYE).

6TH JULY 1988

AUTHORISATION

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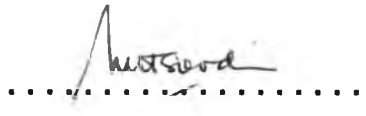

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MA SOODIN.

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ABSTRACT

The use of nucleoside analogues as antiviral and carcinostatic agents is gaining widespread prominence in clinical practices. The introductory sections of this thesis describe their importance. This study considers the attempted synthesis of one such analogue - 2′ [2-amino,3(p-methoxyphenyl)propanamido]-2′-deoxy-N^6 N^6-dimethyladenosine, an isomer of the antibiotic Puromycin, which is a common clinically prescribed antibiotic. The two functional groups present (the sugar and base functions) make the nucleoside a somewhat difficult compound to work with. This is well reflected in the commercial cost of compounds of this type.

This study considers the synthesis of the common clinically used antiviral agent adenosine arabinoside (Ara-A) as the precursor to the synthesis of the target compound. The approaches adopted have been firstly the direct manipulation of the sugar function and secondly the established method of transglycosylation. The former approach has been facilitated by the use of 1,3-dichloro,1,1,3,3-tetraisopropyldisiloxane(TIPDS-Cl_2) as a highly selective protective group and the new reagent,dimethylaminopyridine (DMAP) as a specific hypernucleophilic agent. The synthetic route has been beset by other difficulties, one of which we think might be due to the electrostatic effect of this new protective group not previously investigated. The study considers these problems and the attempts at using different routes to the final synthesis.

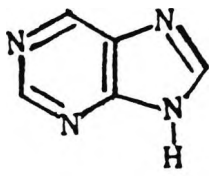
CHAPTER 1

THE NUCLEIC ACIDS

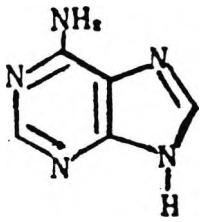
1.1 INTRODUCTION[1].

Nucleoproteins are conjugates of proteins with *nucleic acids*. *Chromosomes* are elongated, sometimes branched structures, composed of nucleoprotein. The material forming the chromosomes is the *chromatin* which is embedded in a matrix. The *nucleolus* and the matrix together form the cell nucleus. The arrangement of nucleoprotein along each chromosome constitutes a store of information, each item of information being located on the chromosome at a definite site called a *gene*. A normal human cell nucleus contains forty-six chromosomes, consisting of twenty-three pairs.

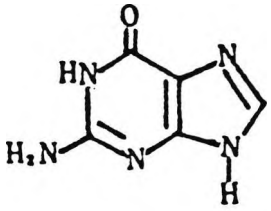
The nucleic acids in chromosomes contain two purine bases, *adenine* and *guanine*, and two pyrimidine bases, *thymine* and *cytosine*. These bases are combined with deoxyribose to form *nucleosides* and then with phosphate to form *nucleotides*. Each nucleotide is linked by its phosphate group to the deoxyribose of the neighbouring nucleotide to form the long chains of *deoxy ribonucleic acid* (DNA). Each chain of DNA contains millions of such units. In addition to serving as a template for its own generation, nuclear DNA also acts as a template for the formation of another nucleotide polymer known as *ribonucleic acid* (RNA).The structure of RNA is basically similar to that of DNA, except that RNA contains the sugar ribose, instead of deoxyribose, and the pyrimidine *uracil* instead of thymine (Figure1.1).



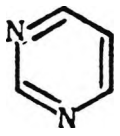
Purine



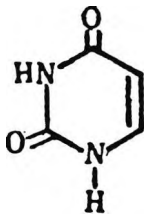
Adenine



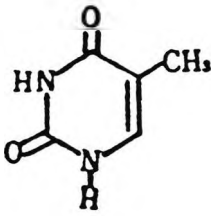
Guanine



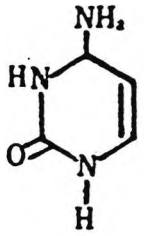
Pyrimidine



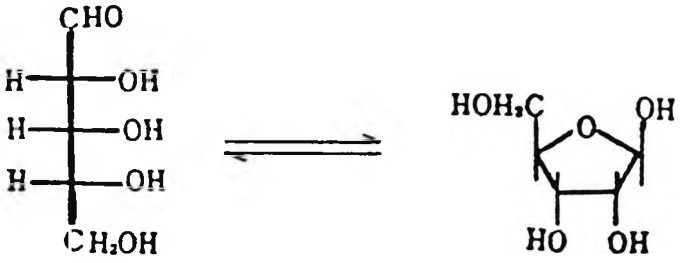
Uracil



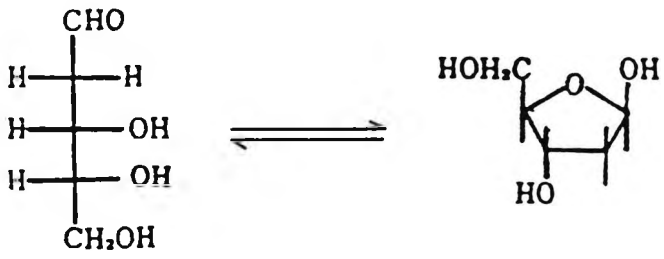
Thymine



Cytosine

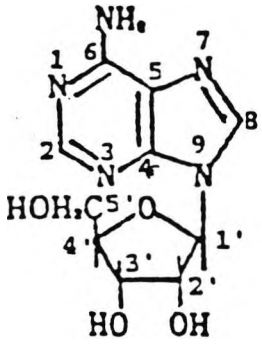


D-Ribose (β-D-ribofuranose)



D-2 -Deoxyribose (β- D-2- Deoxyribofuranose)

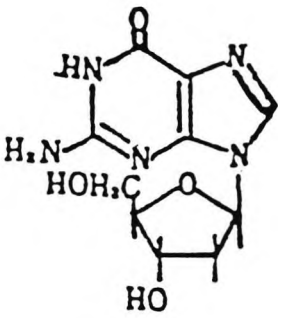
Figure 1.1



9-β -D-ribofuranosyladenine

Adenosine

(RNA Component)

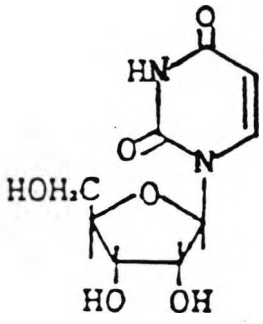


9-β -D-deoxyribofuranosylguanine

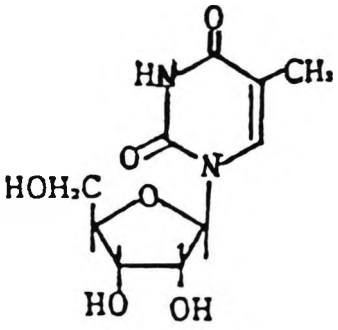
2′-Deoxy- Guanosine

(DNA Component)

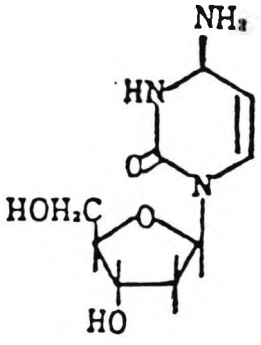
Purine Nucleosides



Uridine

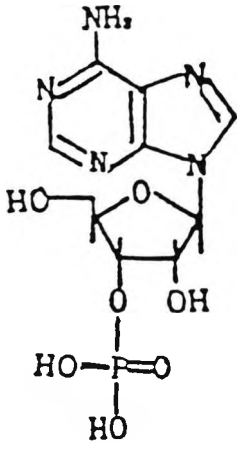


Thymidine



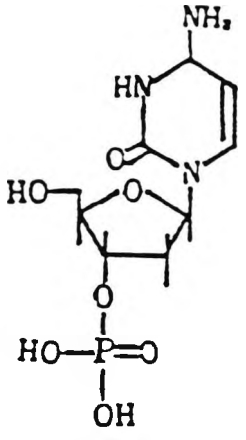
2′-Deoxy-Cytidine

Pyrimidine Nucleosides



3′-Adenosine monophosphate

Purine Nucleotide



2′-Deoxy, 3′-Cytosine monophosphate

Pyrimidine Nucleotide

Figure 1.2

1.2 ISOLATION OF NUCLEIC ACIDS[2].

Because of their tight association with cellular proteins, the isolation of nucleic acids has presented many problems. As for many proteins, the polynucleotides are soluble in dilute salt solutions and are precipitated by the common protein precipitants, trichloroacetic acid or perchloric acid. The procedure most commonly used to isolate RNA is to extract a buffered suspension of the tissue with aqueous phenol. The denatured protein is extracted into the phenol phase or remains at the interface, and the nucleic acids remain in the aqueous phase. The RNA can then be precipitated from this solution by the addition of ethanol. The RNA prepared by this method is a complex mixture and must usually be fractionated before use. These high molecular weight molecules are very susceptible to digestion by various nucleases, and purification is often carried out in the presence of nuclease inhibitors. Crude preparations of RNA are often fractionated on the basis of molecular weight,by centrifugation in a sucrose gradient or, if only small amounts are available,by electrophoresis in polyacrylamide gels.Various column chromatographic methods have also been used to fractionate RNA.

DNA is also obtained by disruption of the protein nucleic acid complex with organic solvents, phenol, or detergents and selectively precipitating the DNA from an aqueous phase. In addition to the problem of nuclease digestion, care must be taken to prevent mechanical shear forces from degrading the extremely long DNA molecules. Different types of DNA molecules

can be separated by centrifugation through gradients of sucrose or caesium chloride, or by chromatography.

1.3 VIRUSES[2].

The most extensively studied class of nucleoproteins are the small infectious particles called viruses. Viruses are parasitic agents and are capable of replication only within a host cell. The basis for the understanding of the chemical nature of the viruses was established in 1935 with the isolation and crystallisation of the tobacco mosaic virus by Stanley[2,90]. He was able to demonstrate that this virus consists of a single ribonucleic acid molecule surrounded by protein subunits, which form a protective coat. The infectivity of the virus is a function of the nucleic portion only, and the protein serves as a protective device to stabilize the mature viral particle and, in some cases, to aid in the process of infection.

1.4 THE CHEMISTRY OF NUCLEIC ACIDS.

The complete hydrolysis of a nucleic acid preparation yields phosphate, a sugar, a pair of pyrimidine bases, and a pair of purine bases. Both of the sugars found in nucleic acids are D-pentoses, and both are present in the beta-furanose form. The bases commonly found are the three pyrimidines - cytosine, thymine and uracil; the two purines bases are adenine and guanine. Those bases containing exo-ring oxygen atoms usually undergo tautomerisation between the keto and the enol form. The predominant form in this equilibrium is pH dependent. At physiological values of pH, the keto form tends to predominate

in the case of the free base. Similarly in the nucleic acid molecule, the chemical binding to the ring nitrogen atom also pushes the equilibrium to the keto form.

High molecular weight DNA and RNA differ considerably in their susceptibility to hydrolysis under alkaline conditions. Although DNA is rather stable to dilute basic conditions, RNA is easily broken down to the nucleotides. Nucleotides contain one mole each of ribose, a purine or a pyrimidine, and a phosphate. In the nucleotide unit, the ribose is joined to the purine or pyrimidine by an N-glycoside bond to the N-9 of a purine or N-1 of a pyrimidine and the phosphate is present as an ester on the 3^{1} or 5^{1}-hydroxyl group of the ribose. It can be further degraded by alkaline hydrolysis to a phosphate and a nucleoside. The latter consists of a pyrimidine or purine base attached to a sugar unit. Alternatively, acid hydrolysis will convert the nucleotide to a sugar phosphate and the free base (Figure 1.2, page 3).

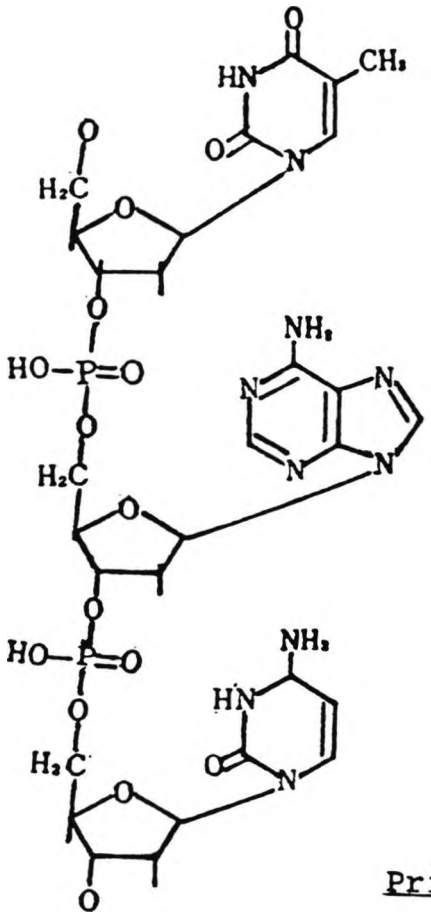
1.5.1 THE PRIMARY STRUCTURE OF NUCLEIC ACIDS.

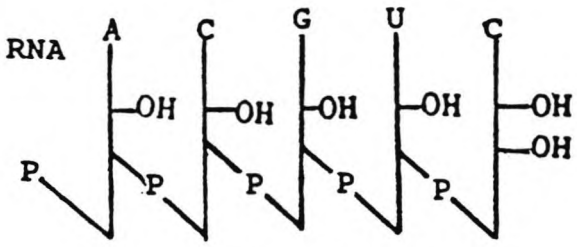
The primary structure of the nucleic acids has been determined mainly by the use of specific enzymatic and acid or base-catalysed hydrolytic procedures. The high molecular weight nucleic acid molecules were cleaved into smaller fragments, and the knowledge of the structure of these was used to determine which chemical linkages were present in the parent molecules. The basic structure is composed of a series of nucleosides held together in a long linear chain by a phosphodiester linkage

from the 3′-hydroxyl group of one sugar to the 5′-hydroxyl group of the next. It should be noted that an alternating phosphate-sugar-phosphate-sugar chain forms this structure, and that the nitrogenous bases are simply attached to this sugar backbone. A convention has been accepted to illustrate the shorthand designations commonly used (Figure 1.3). The convention is such that the end containing a 5′-OH or 5′-phosphate group, or with both 3′-and 5′-positions unesterified,is written to the right. These shorthand formulae for oligonucleotides make it possible to discuss efficiently and unambiguously different sequences of nucleic acid segments without involving a cumbersome structural formula.

1.5.2 THE SECONDARY STRUCTURE OF NUCLEIC ACIDS.

The key observation which allowed the correct postulation of the structure of DNA was made in 1953 by Watson and Crick[3], who were working in Cambridge. They demonstrated that it was possible to arrange the pairs of bases, adenine and thymine and guanine and cytosine in such a way that hydrogen bonds were readily formed between them. The hydrogen bonds are formed between a pair of electrons on the keto group or ring nitrogen of one base and a hydrogen atom on a ring nitrogen or amino group on the other (Figure 1.3). The two base pairs which are formed have very similar dimensions and allow the construction of a double helical structure where the bases extend to the inside of the helix and hold it together by their hydrogen-bonded interactions. Although an individual hydrogen bond is very weak, the large number of them formed in the DNA molecule

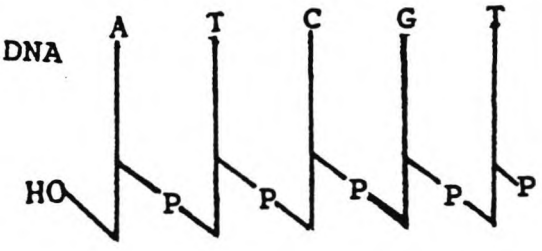




pApCpGpUpC

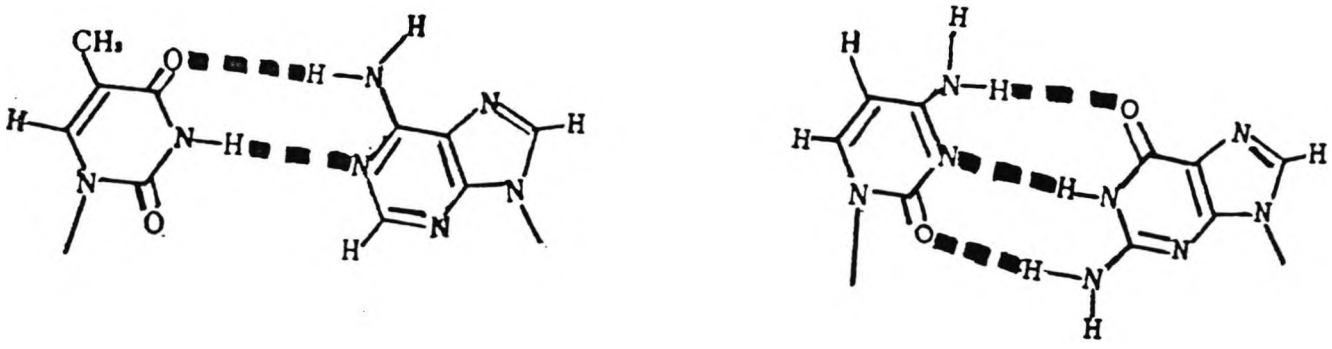
Two ways of designating the structure of oligonucleotides.

dApdTpdCpdGpdTp



Primary Structure of DNA

Segment of a DNA molecule showing phosphodiester bonds between nucleotide units.



Secondary structure of DNA.

Hydrogen bonding between the adenine-thymine and guanine-cytosine bases in DNA. The A:T pair has two hydrogen bonds; the G:C pair has three.

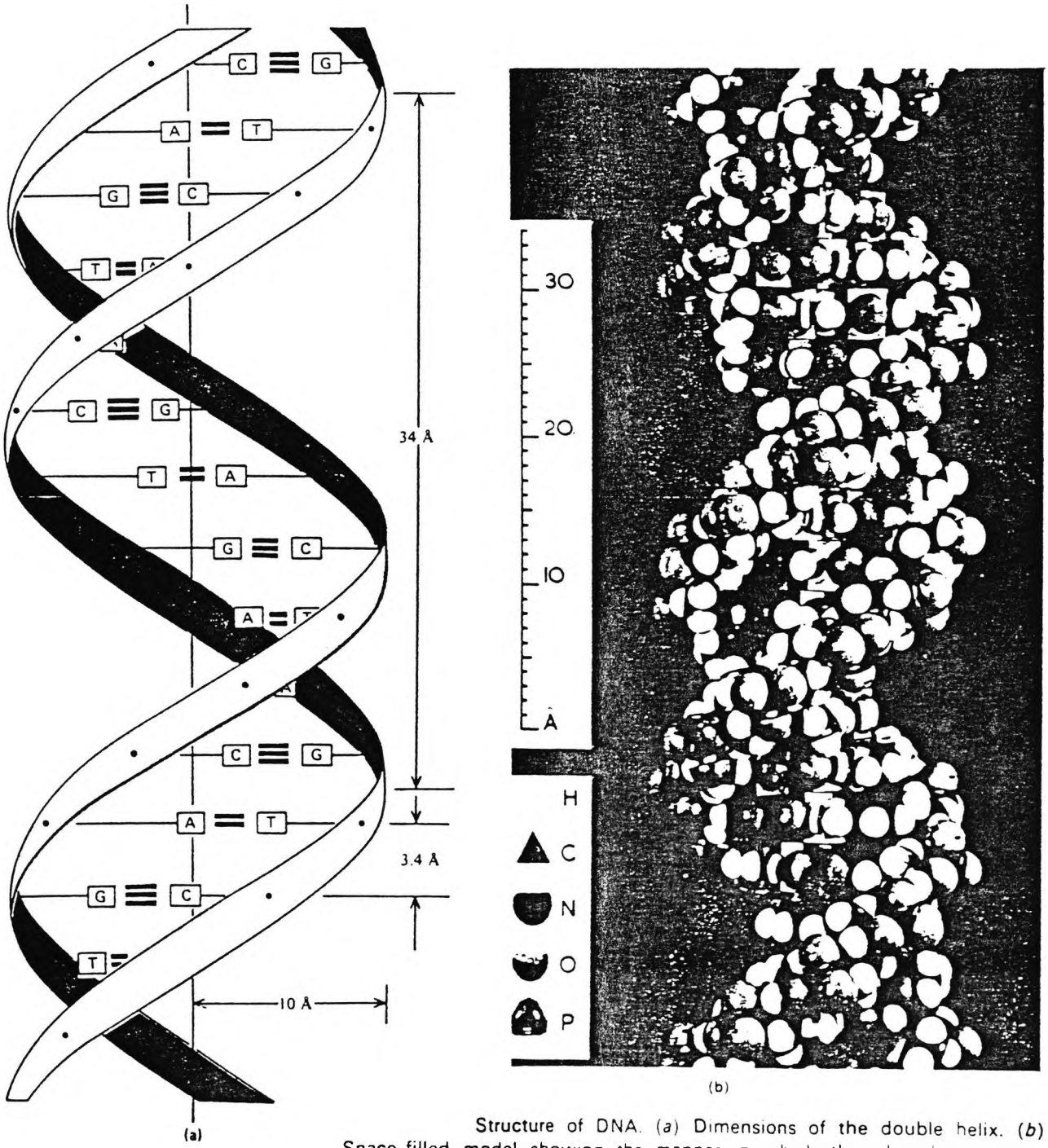
Figure 1.3

effectively stabilizes the structure. The structure is also stabilised by hydrophobic interactions between the stacked base pairs.

X-ray studies of the base pairs showed that the distance involved between the base pairs is consistent with the distance covered by a single twist of a helix. The structure required that the two strands of DNA run in opposite directions, i.e the direction from the 3′ and 5′-phosphates was different, but it posed no restriction on which bases might be present at any point along the chain. An A:T pair had essentially the same dimension as a G:C pair (Figure 1.4). It was clear to Watson and Crick[3] at the time the structure was proposed that the specific base pairing involved in stabilizing the structure of DNA could be accurately duplicated during cell division, and by which a DNA of unique base sequence could be passed from a mother to a daughter cell. Although other types of base pairing have been suggested, the currently accepted model for the structure of DNA is essentially the same as that originally proposed. It has also been possible to prepare synthetic DNA-like polymers whose properties are those predicted by the model.

1.5.3 THE TERTIARY STRUCTURE OF NUCLEIC ACIDS.

Cellular chromosal DNA cannot be isolated in the form of an intact molecule. Because of its large size, the chains are broken by shear forces generated in the preparations, or they are cleaved by nucleases in the preparation. Much more



Structure of DNA. (*a*) Dimensions of the double helix. (*b*) Space-filled model showing the manner in which the phosphate groups point to the outside of the helix and the bases to the interior. (Professor M. H. F. Wilkins, Medical Research Council, King's College, London).

(H.R Mahler, E.H Cordes, 'Basic Biological Chemistry' p. 122-123, Harper & Row, New York and London, 1968).

Figure 1.4

information is available however, regarding some of the smaller DNA molecules.

Some viruses contain double-stranded linear DNA molecules which behave as long, stiff, worm-like coils in solution. These give the DNA its characteristic viscosity. Probably the most extensively studied DNA molecules are the double-stranded cyclic DNA molecules found in bacteria, mitochondria, chloroplasts, and some viruses. These molecules can form into a superhelical structure by twisting the circular molecule, or under some conditions they can collapse into a relatively unstructured coiled ball. Examples of single-stranded linear and single-stranded cyclic DNA preparations are also known.

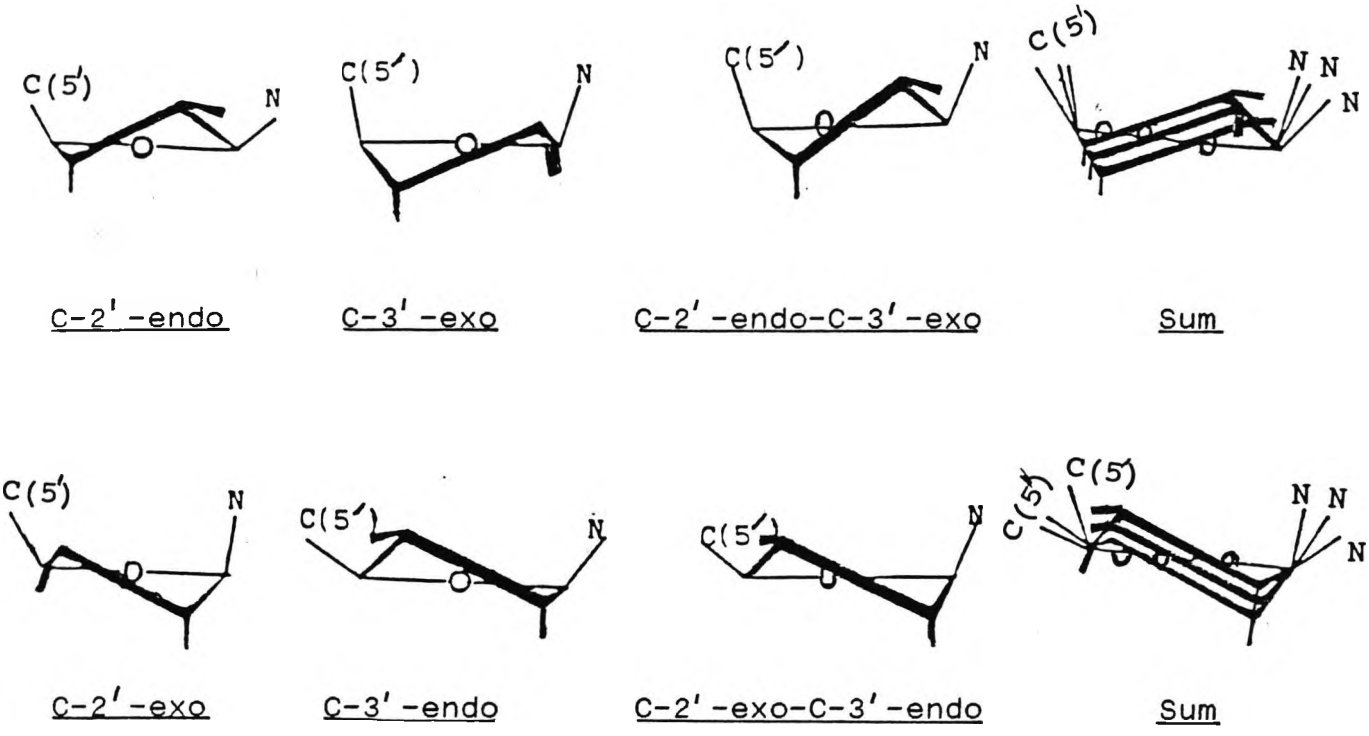
1.6. THE CONFORMATIONS OF NUCLEOSIDES AND NUCLEOTIDES[4,5].

The structure of nucleosides and nucleotides allows them to adopt a number of conformations depending on (1) the sugar ring puckering, (2) the orientation about the glycosidic bonds and (3) the position of the O-5′ atom. Each of these aspects is considered in turn below.

1.6.1 SUGAR RING PUCKERING.

The five-membered sugar ring can adopt two puckered forms: a twist (half-chair), T, or an envelope, E, form. Although the pucker moves around the sugar ring, the preferred puckering modes involve the C-2′ and the C-3′ atoms and are shown in Figure 1.5.

Figure 1.5 Preferred Sugar Ring Puckering Modes.



C-2'-endo means that the C-2' atom is out of the plane described by the C-1', C-3', C-4', and O-1' atoms by about 0.5Å and on the same side as C-5'. In C-2'-exo, the C-2' atom is on the opposite side of the plane with respect to the C-5' atom. It should be noted that for each ring pucker there is an associated orientation of all the exocyclic bonds. Energetically, the conformations shown along a horizontal line (refer to diagram) are close, whereas there is an energy barrier of ca. 5 kcal/mole separating the two sets of conformations. The whole range of sugar ring conformations has been described by Altona and Sundralingham[6] who make use of a psuedorotation cycle.

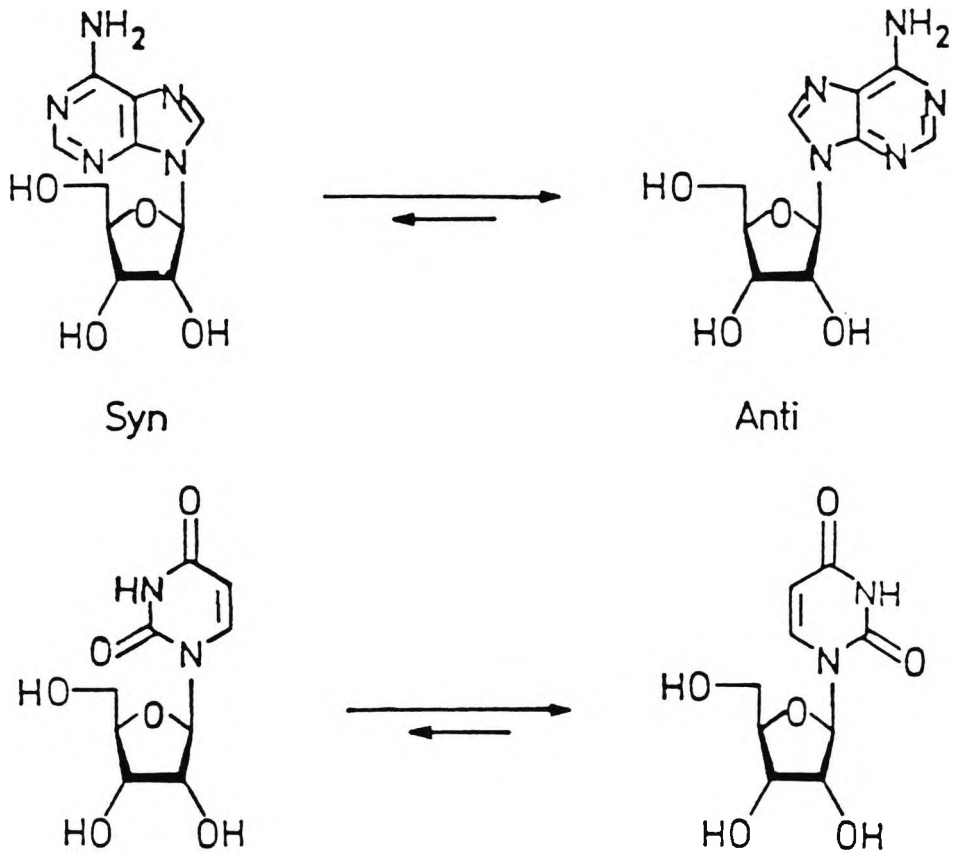
In solution, nucleotides display a C-2'-endo $\rightleftharpoons$ C-3'-endo equilibrium[4]. However, in DNA and RNA, the double-helical

structure imposes constraints on the sugar conformations. The B-, and C-DNA display a C-3 -exo ring pucker[7]. In RNA however, the C-3′-endo ring pucker is the most commonly found[8].

1.6.2 ORIENTATION ABOUT THE GLYCOSIDIC BOND.

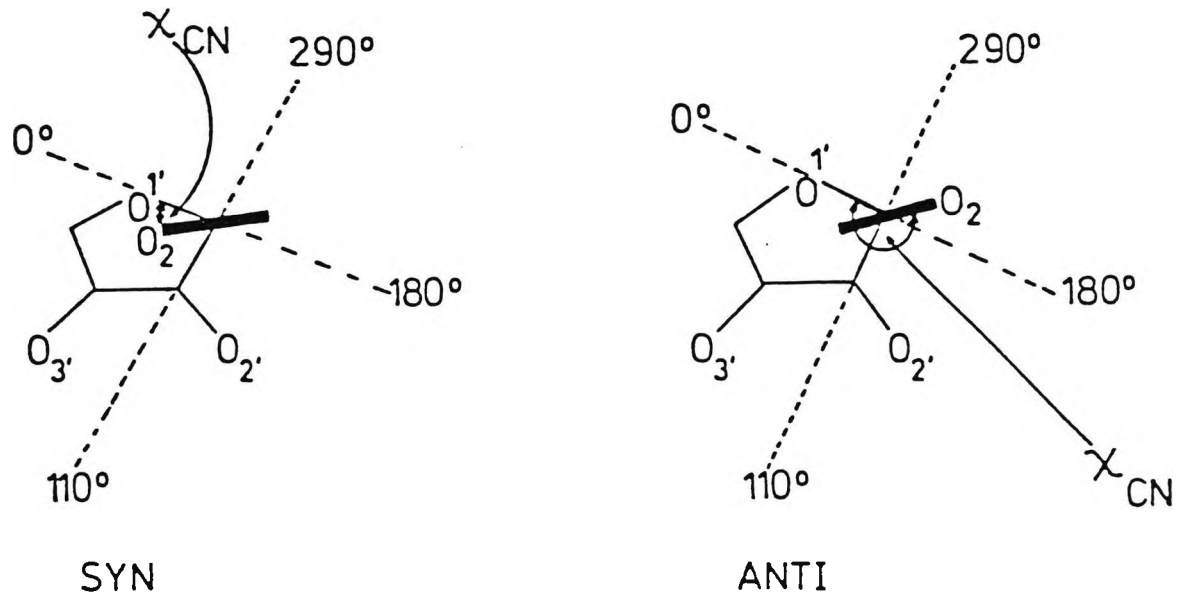
The base in a nucleotide generally adopts one of two conformations. These are known as *syn* and *anti*, roughly depending on whether the oxygen atom attached to C-2 in pyrimidine nucleotides or the nitrogen atom at the 3-position in purine nucleotides, is above the sugar ring or pointing away from it (Figure 1.6).

Figure 1.6 Orientation about the Glycosidic Bond.



The position of the base is given by the dihedral angle X_{C-N} as defined in Figure 1.7. Thus the syn and anti conformations are defined by values of X_{C-N} between 290 - 110° and 110 - 290° respectively. Although the two conformations exist in dynamic equilibrium in solution, the anti conformation is preferred in both purine and pyrimidine nucleotides[7]. However, the syn orientation is preferred in some 6-substituted pyrimidine nucleotides and 8-substituted purine nucleotides[9,10].

Figure 1.7 Syn and Anti Orientations in Pyrimidines.

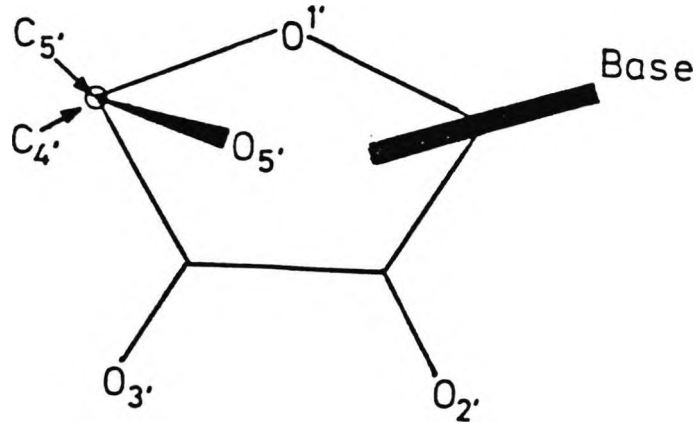


1.6.3 ORIENTATION ABOUT THE C-4' - C-5' BOND.

The orientation about the C-4' - C-5' bond is such that the O-5' atom is above the plane of the sugar ring (Figure 1.8) and can form hydrogen-bonding interactions with the heterocyclic ring. Although many exceptions to this rule occur in nucleosides,

most 5-nucleotides show preference for this orientation[11], which is the one that occurs in double-stranded DNA.

Figure 1.8 Position of the O-5' Atom.



CHAPTER 2

INHIBITORS OF NUCLEIC ACIDS SYNTHESIS[12,13].

2.1 INTRODUCTION.

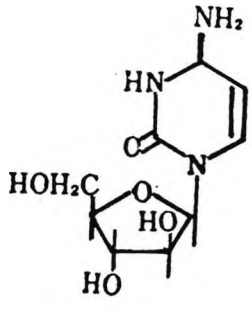
Antibiotics and drugs may interfere with the synthesis of nucleic acids in several ways. It is convenient to define three levels at which inhibitors may exert their primary effect, and to arrange the known antimicrobial agents into three groups. First there are those substances whose ability to inhibit nucleic acids synthesis is exerted at the level of nucleotide metabolism, usually by blocking the synthesis of nucleotides or by deranging the delicate balance of reactions concerned with interconversion of nucleotides. The second group are agents which act at the level of the polymerisation reactions. These agents affect the DNA molecule directly, resulting in the impairment of its capacity to function as a template for the polymerisation reactions. Thirdly, there are those agents which inhibit nucleic acids synthesis via direct interference with the polymerases or other enzymatic processes involved in the replication and transcription of DNA.

Our study considers one group, the action of which causes disruption to the DNA molecules to act as a template for the polymerisation reactions. Its mode of action involves the inhibition of the utilisation of nucleotides. As a result these agents have found popularity in present day cancer chemotherapy because they provide a means of discriminating selectively against fast-growing tumour cells.

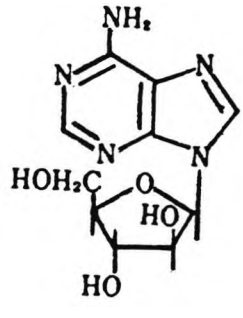
2.2 ARABINOSIDES AS INHIBITORS OF NUCLEOSIDE UTILISATION[14].

Only a few agents can be tentatively assigned into this category, for it can fairly be argued that the postulated existence of 'pure' inhibitors of nucleoside utilisation may be spurious. The most important of the groups are the two arabinose-containing nucleoside analogues: cytosine arabinoside (Ara-C) and arabinosyladenine (Ara-A) (Figure 2.1). The former is a synthetic antitumour drug; the latter occurs naturally as a metabolite of *Streptomyces antibioticus*. Great interest attaches to Ara-A as an antiviral agent and more recently it has attracted attention as an anticancer drug of growing importance, largely through the discovery that its inactivation by adenosine deaminase in mammals can be blocked by deaminase inhibitors such as deoxycoformycin (itself a nucleoside antibiotic analogue of adenosine) which thereby greatly potentiates the cytotoxic action of Ara-A. Although the anti-cancer nature of Ara-A has been firmly established, its use in the treatment of cancer is still uncommon. However, its use as an antiviral agent is well established in present day clinical treatment, especially in life-threatening conditions.

Figure 2.1 Chemical structure of Ara-C and Ara-A.



Cytosine Arabinoside (Ara-C).



Adenine Arabinoside(Ara-A).

2.3.1 ADENINE ARABINOSIDE[15].

Adenine arabinoside (9-β-D-arabinofuranosyladenine; vidarabine; adenosine arabinoside; Ara-A) is a purine nucleoside analogue, which was originally synthesised as a potential anti-cancer agent. It was subsequently discovered to be a naturally occurring nucleoside present in culture filtrates of *Streptomyces antibioticus*. Adenine arabinoside has a broad antiviral spectrum, but it is primarily active against the herpes and pox viruses. By comparison to the pyrimidine analogues, idoxuridine and cytarabine, it has a low toxicity, its primary metabolite has high antiviral activity, it has a lower immunosuppressive effect and it affects rapidly dividing cells to a lesser extent. It is of proven value in certain viral eye infections, but its place in other viral infections has yet to to be determined by controlled trials.

Another drug,adenine arabinoside 5'-monophosphate, which is the nucleotide precursor of adenine arabinoside, is being studied. It has the advantages of being highly soluble in water and of being broken down in man, resulting in sustained serum and tissue levels of fully active drugs[15].

2.3.2 ANTIVIRAL ACTIVITY[15].

In 1968 it was reported[16] that adenine arabinoside was active *in vitro* against *Herpesvirus hominis* and *Vaccinia virus*. Subsequently it was shown to be active *in vitro* against other DNA viruses such as *Herpesvirus varicellae* and *Cytomegalovirus* and a single RNA virus (*Rous sarcoma*). This activity, which

has been confirmed in a variety of different types of cell culture, has also been extended to include other poxviruses such as Monkeypox and Tanapox and strains of *Herpesvirus hominis* types 1 and 2, which are resistant to idoxuridine. It is doubtful whether adenine arabinoside has any activity against human adenoviruses. The drug has no activity against non-oncogenic RNA tumour viruses, but it has a fairly broad spectrum of activity against oncogenic RNA tumour viruses. The previous problem of the rapid conversion of adenine arabinoside to Ara-inosine (Ara-I) by adenosine deaminase in cell cultures has been overcome by the inhibitor deoxycoformycin, thus maintaining the minimum inhibitory concentration values against these viruses. This has led to the development of adenine arabinoside as the leading nucleoside antibiotic.

The activity of adenine arabinoside has also been extensively studied in a variety of experimental viral infections of animals. It has antiviral activity against *Herpesvirus hominis* and vaccinial keratitis, comparable to that obtained with idoxuridine, and it is also effective against skin lesions produced by these viruses. Adenine arabinoside increases the survival rate of animals with experimental *Herpesvirus hominis* and vaccinal encephalitis.

Depending on the experimental animal model studied, adenine arabinoside is effective when administered intraperitoneally, subcutaneously, perorally, intravenously, subconjuctivally, intracerebrally or by topical application. For a given chemo-

therapeutic effect, it has much less toxicity than either idoxuridine or cytarabine. In some animal experiments, adenine arabinoside only reduced viral replication sufficiently to allow normal host defence mechanism to overcome the infection. In this context it is of interest that adenine arabinoside and human interferon are synergistic against *Herpesvirus hominis* type 1 in tissue culture. Also when it is used with humoral antibodies to *Herpesvirus homonis*, there is enhanced protection against *Herpesvirus homonis* in mice. These findings support the view that drugs such as adenine arabinoside can only reduce viral load in an acute illness, thereby assisting the host immune mechanisms to produce the final cure. This also explains the failure of the drug for the treatment of some viral infections in hosts with defective immune responses.

2.3.3 MODE OF ACTION OF ADENINE ARABINOSIDE[15].

Adenine arabinoside selectively inhibits viral DNA synthesis but the exact biochemical mechanism is still unknown. It has been suggested that its triphosphate metabolite may inhibit viral DNA polymerase or virus-induced ribonucleotide reductase. *In vitro* adenine arabinoside triphosphate also inhibits mammalian cell DNA polymerases but to a lesser extent than its inhibition of viral DNA polymerase. It can also be incorporated into both cellular and viral DNA during DNA synthesis. Other studies indicate that both adenine arabinoside (vidarabine) and cytidine arabinoside (cytarabine) may act by a similar mechanism, in that the triphosphate derivatives of both drugs have DNA polymerase as their primary target.

2.3.4 CLINICAL USES OF ADENINE ARABINOSIDE[15].

The following is a summary of the cumulative evidence based on carefully planned trials, particularly in the United States, which defines the place of this drug in antiviral chemotherapy.

1.Ocular infections[17]. The efficacy of adenine arabinoside as compared to idoxuridine (IDU) has been studied in a double-blind trial in which both drugs were used topically to treat human keratoconjunctivitis. Adenine arabinoside (Ara-A) was as effective as IDU for treatment of *Herpesvirus hominis* keratis. It was also a suitable non-toxic alternative for treatment of cases resistant to IDU or with allergic or toxic reactions to IDU. Ara-A was as effective as IDU in the presence of corticosteroid medication for deep herpic disease. Like IDU, Ara-A was of no value for adenoviral keratoconjunctivitis. Further comparisons between IDU and Ara-A have been made in the treatment of herpeic uveitis. Ara-A was only of value in those patients with defective corneal epithelium because this enabled its metabolite ara-inosine (Ara-I) to enter the eyes; IDU was of no value, regardless of the corneal status, because although its metabolite uracil also penetrated the eyes when the cornea was disrupted, it has no antiviral activity. Adenine arabinoside (Ara-A) has also been given intravenously in a dose of 20mg per kg per day to patients with herpetic keratouveitis. It was effective because the drug penetrated into the anterior chamber and this treatment was accompanied by only minimal side effects.

Numerous other studies have confirmed the value of Ara-A in

acute or recurrent herpes keratitis; it is as effective as IDU and preferable to that drug because of the intraocular penetration of its metabolite, its lack of normal tissue toxicity and its lesser allerginic effect. Also clinical resistance to the drug is very rare. Ara-A also appears to be the prophylactic drug of choice in corneal transplant patients subject to cold sores and other herpetic lesions, because it results in healthier epithelial healing and is no more detrimental than IDU in slowing stromal repair.

2.*Herpesvirus Varicellae* Infections[18]. Adenine arabinoside appeared to be useful for the control of localised herpes zoster and also for the disseminated disease in immuno-compromised patients; it did not seem to influence zoster encephalitis or complicated variecella in immuno-compromised patients. The results of a controlled study of the use of intravenous Ara-A to treat herpes zoster in immuno-compromised patients were as follows: in spite of rapid natural healing of both localised and disseminated disease, patients who received Ara-A for the first five days had more rapid clearance of virus from vesicles and cessation of new vesicles formation and the time taken to reach the vesicular stage was shortened. It was concluded that the drug is most efficacious when it is administered during the first six days of the disease and when the patient has reticuloendothelial neoplasia and is aged less than 38 years.

3.*Herpesvirus Hominis* Infections.

(a) Encephalitis[19]. To avoid the many biases associated with uncontrolled studies of antiviral drugs in this disease, a

collaborative controlled study of the use of Ara-A in virologically confirmed *Herpesvirus hominis* encephalitis was instituted in the United States. In confirmed cases of the virus by brain biopsies, there was a 70% reduction in mortality among the children under investigation. Treatment was not effective in children in whom the virus was not detected by brain biopsies. Recovery by the children whose infection was treated with Ara-A had varying degrees of mental impairment.

(b) Mucotaneous infections[20]. Ara-A has been used to treat patients with severe mucotaneous infections, most of whom were receiving immunosuppressive therapy. It appeared that the drug was effective in intravenous doses, particularly for lesions due to *Herpesvirus hominis* type 1.Clinical improvement was parallelled by a reduction in virus excretion from the throat and clinical lesions. Excretion of virus returned in some patients weeks after cessation of therapy, suggesting that the drug only reduces viral replication and recovery depends on the integrity of the host's immune system. Three patients with concomitant genital lesions due to type 2 virus showed no favourable response.

Topical treatment with 3% Ara-A has been studied in a controlled trial involving 55 men and 42 women with genital herpetic infection. Treatment did not influence the course of either primary or recurrent genital infection.

(c) Neonatal Infections[21]. Ara-A has been used to treat neonates with *Herpes hominis* infection, some with disseminated disease. The survival rate was 61% and none had any

neurological deficit after a year.

4.*Cytomegalovirus* Infections[22]. Ara-A has not been beneficial in the treatment of neonates with disseminated congenital infection, nor has it been beneficial to adults with disseminated infection in renal transplant recipients on immunosuppressive therapy. However, with adults who were immunologically normal, intravenous therapy with Ara-A has given a complete success rate.

5.Smallpox[23]. A controlled study of the efficacy of Ara-A in the treatment of variola major was conducted in Bangladesh. The results showed that the drug was ineffective in the treatment of smallpox.

6.Chronic Hepatitis B[24]. Intravenous therapy of Ara-A to patients with chronic hepatitis B has shown good response. Recovery was complete with no residual liver dysfunction as confirmed by liver function tests.

2.4 PUROMYCIN-AN ANTIBIOTIC INHIBITOR OF RIBOSOME FUNCTIONS[13].

Following the initial suggestion[25,26] that antibiotics might exert highly specific inhibitory effects in sensitive cells, many such compounds (e.g puromycin - Figure 2.2, page 27) have been shown to selectively inhibit protein synthesis. Among the antibiotics which act in this way, many show selective toxicity, in that they are active against bacteria but not against eukaryotes (or vice versa), whereas others inhibit growth of a wide range of both pro- and eukaryotic cells. By far the majority of antibiotics which act against protein synthesis do so by inhibiting functions of the ribosomes, the

multi-macromolecular complexes on or in which decoding of the genetic message occurs. Studies of the actions of ribosome function inhibitors have also contributed enormously to our knowledge of the structure and function of ribosomes and of polypeptide biosynthesis. Also, if we are to design and exploit new drugs at some future date it is vital to understand how currently available antibiotics are able to discriminate between structures and processes in different cells.

Since it became clear that proteins are synthesized *in vivo* on ribonucleoprotein particles, subsequently termed 'ribosomes', these structures have been subjected to intense chemical and physical analysis[27,28]. It soon became apparent that ribosomes from bacteria can be readily distinguished from those of higher organisms on the basis of sedimentation coefficients ('S values'). From this grew the practice of using S values as proper nouns and of referring to '70S' ribosomes of prokaryotes and '80S' ribosomes of eukoryotes. Although these nominal S values hide a multiplicity of sedimentation coefficients, this convention is particularly useful in the context of antibiotic action since many inhibitors discriminate between the types of ribosome[29,30]. Many antibiotics thus show selective toxicity by the types of ribosome (70S or 80S) involved. Puromycin shows specific toxicity towards 70S/80S ribosomes[31,32].

2.5 THE MODE OF ACTION OF PUROMYCIN[13].

Clarification of the mechanism of peptide-bond formation on ribosomes was intimately associated with the elucidation of the

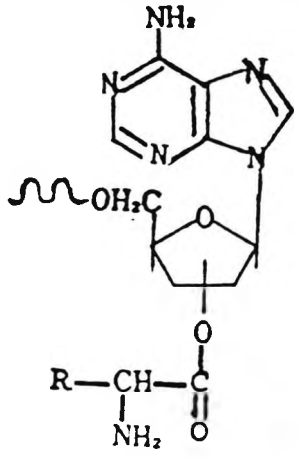
mode of action of the antibiotic, Puromycin (Figure 2.2), and with consideration of the so-called ' Puromycin reaction '. This drug has occupied a central role in many studies, both of protein synthesis and of the inhibition of ribosome function by other antibiotics. Puromycin also affords the only clear example of how the structure of a ribosome-inhibitor is related to its inhibitory action.

Puromycin is a structural analogue of aminoacyl-adenosine, the 3′-terminus of aminoacyl-transfer RNA (*t*RNA)[33] (Figure 2.2). When the antibiotic was added to intact reticulocytes, nascent globin chains were removed from ribosomes[34] and it was later shown that poly-phenylalanine was released from ribosome-bound *t*RNA when puromycin was added to extracts of *E.Coli* supplemented with polyuridylic acid[35]. Next came the notion that at least some of the peptides released from ribosomes by puromycin have the drug molecules covalently attached to them.

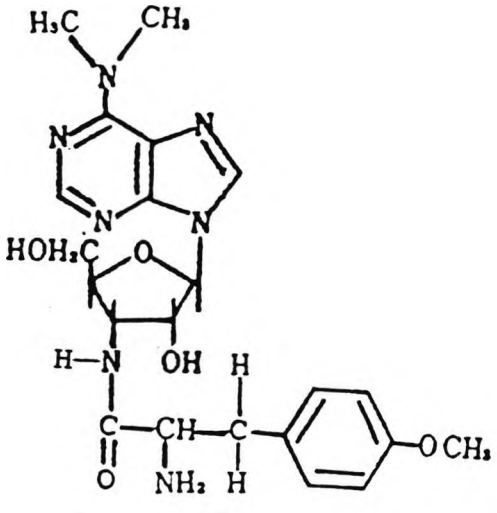
The relationship between this so-called ' Puromycin reaction ' and ribosomal peptide bond formation was investigated in a classic series of experiments by Traut[36] (1964). He demonstrated that the 70S (the 'S value' the Sedimentation Coefficient, is a basis for distinguishing bacteria from those of higher organisms) ribosomes of E.Coli-bearing polyphenylalanine-*t*RNA could be removed from a 'polyuridylic system ', washed free from other cell components, and would then subsequently react with Puromycin to form polyphenylalanine-puromycin. This was the first demonstration that ribosomes

could catalyse the formation of peptide bonds in the absence of cytoplasmic factors and without any added energy source {eg guanosine triphosphate (GTP)}. Traut[36] did, however, find greater reactivity with Puromycin in the presence of guanosine triphosphate (GTP) and cell supernatant. Accordingly it was suggested that ribosomes bearing peptidyl-*t*RNA can exist in two states, only one of which allows direct reaction of peptidyl-*t*RNA with Puromycin. The relationship between the two ribosomal states and the modern terminology involving A and P sites is obvious; peptides in the P sites are reactive and those in the A sites are non-reactive towards puromycin[35,36].

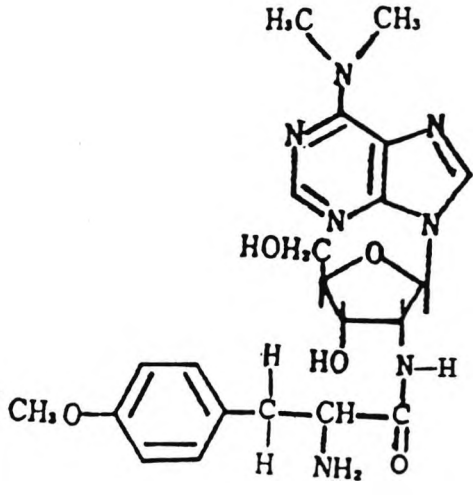
Figure 2.2 Structure-Relationship Of Transfer RNA-Amino Acid To Puromycin and Target Compound.



tRNA



Puromycin



Target compound

2.6 RELATIONSHIP OF THE PUROMYCIN REACTION TO PROTEIN SYNTHESIS[13].

Because of the marked similarities between the Puromycin reaction and the ribosomal peptide bond-forming reaction, the puromycin reaction is a useful model system for studying the synthesis of individual peptide bonds. Both processes require Mg^{++} and K^{+} (NH_4^{+}) ions, both are peptidyl transfer reactions, and the substrate specificities at both the P site and the A site are similar in both cases. Thus peptides or aminoacids occupying the P site and the A site are also similar in both cases. Peptides or amino acids occupying the P sites are much better substrates if they have blocked amino groups[37] and for reactivity they must be attached to *t*RNA or an oligonucleotide fragment. Finally, many antibiotics which inhibit protein synthesis also inhibit the Puromycin reaction in one of its many forms. A better argument is the converse one; all compounds known to inhibit Puromycin reactions can be shown to inhibit protein synthesis under similar conditions[38].

As a result of these and other observations, it is now generally accepted that Puromycin acts as an analogue of aminoacyl-*t*RNA bound in the ribosomal A site, takes part in the ribosomal peptide bond-forming reaction and accepts the nascent peptide chain. Since Puromycin binds only weakly to ribosomes, the resultant peptidyl-puromycin molecule usually falls off the ribosome almost immediately.

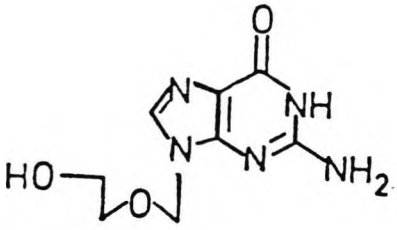
2.7 OTHER GROUPS OF NUCLEOSIDES WITH BIOLOGICAL ACTIVITIES.

The biological activities and medical applications of the arabinosides, the 5′-substituted-2′-deoxy-ribofuranosyl-nucleosides, eg. Ara-A and Ara-Cytidine as discussed above, are well established. It is worth noting at this point that there are other groups of nucleosides which have proven medical applications, as described in the following sections.

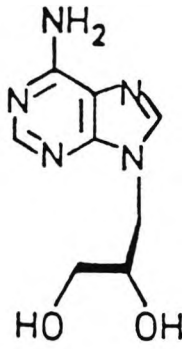
2.7.1 ACYCLIC NUCLEOSIDES.

This class of compounds does not have a sugar ring attached to the base. In its place there is a short chain which in certain conformations is identical to parts of the sugar ring that it replaces. A number of these types of compounds have potent and selective antiviral properties. Three of these compounds, (R)-9-(3,4-dihydroxybutyl)guanine (DHBG), 9-(2-hydroxyethoxy-methyl)guanine (acyclovir, ACV) and 9-(1,3-dihydroxy-2-propoxy-methyl)guanine (DHPG) (Figure 2.3) seem to have a common mode of action. ACV, presently marketed under the name acyclovir, is a widely prescribed drug used mainly for the systemic and topical treatment of viral and herpes infections. There is no firm evidence at the present that the remaining ones, as for example, 9-(2RS,3SR,dihydroxy-2-propoxymethyl)adenine (DHPA) (Figure 2.3) have any medical applications. The compounds are firstly phosphorylated by viral thymidine kinase[39] (TK) and, after conversion to the triphosphates, they inhibit DNA synthesis only in infected cells. This field is currently receiving much attention[39-41].

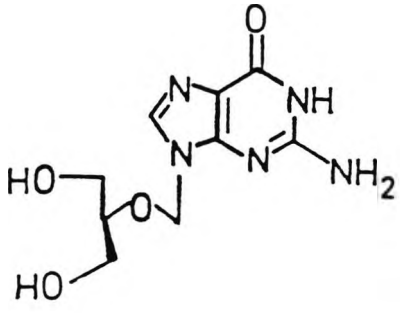
Figure 2.3 Acyclic Nucleosides.



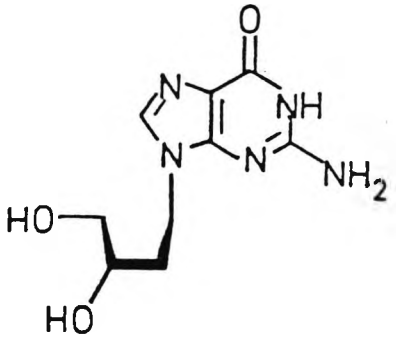
ACV



DHPA



DHPG

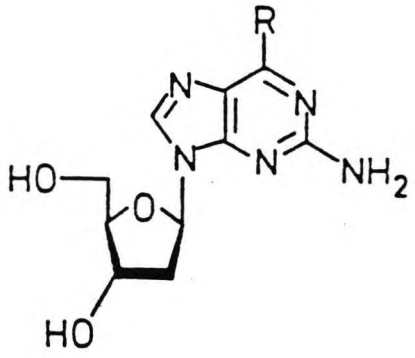


DHBG

2.7.2 NUCLEOSIDES CONTAINING MODIFIED PURINES.

A variety of modifications to the purine bases in nucleosides have been made. Thus compounds in which a nitrogen atom is replaced by a carbon atom and vice versa have been prepared (Figure 2.4). Many thiopurines have also been prepared (Figure 2.5). These compounds were quite popular medically when nucleosides analogues were introduced for cancer chemotherapy, but their medical uses have been discontinued as better and more effective nucleosides have been made available on the market.

2.4 Nucleosides with modified purine bases.



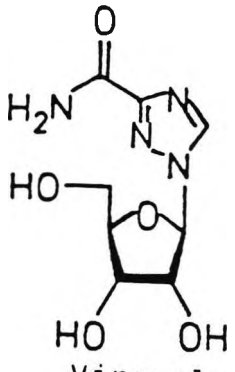
R =

SH

SMe

$SCH_2CH=CH_2$

$NH.NH_2$



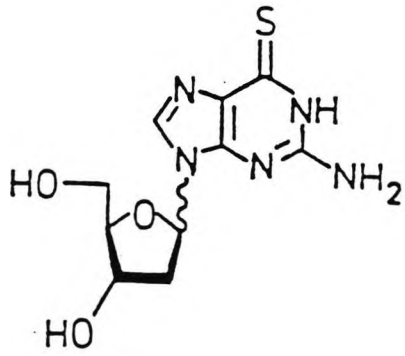
Virazole

These types of compounds generally affect the de novo synthesis of purine nucleotides and are therefore not selective antiviral agents but are more useful as anti-cancer agents. For example, ribavirin has been shown to act at two levels. Firstly, it inhibits the formation of guanosine 5′-monophosphate (GMP) from inosine 5′-monophosphate (IMP)[42]. Secondly it can be phosphorylated, and its triphosphate inhibits influenza virus RNA polymerase[43].

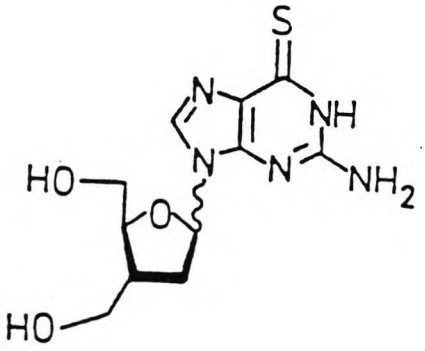
Compounds of interest from one class of nucleosides (the mercaptopurines) are the pairs of anomers of I and II[44] (Figure 2.5). The anomers Iα and Iβ are both phosphorylated in cancer

cells and are incorporated into DNA where they stop further chain elongation. However, the more active anomer Iβ is more toxic. By synthesising the 3'-hydroxymethyl pair of anomers, IIα and IIβ, two compounds of similar activity to Iβ were obtained, but with lower toxicity.

Figure 2.5 Mercaptopurine nucleosides compounds.



I α and β

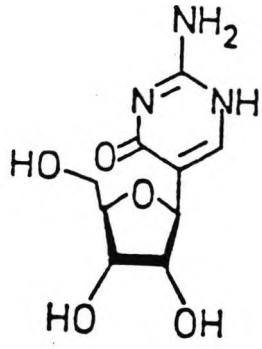


II α and β

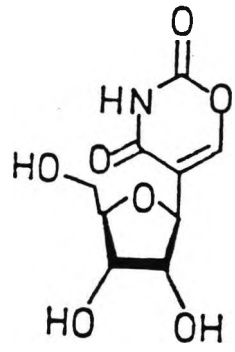
2.7.3 C-NUCLEOSIDES.

This class of nucleoside analogues has the base and sugar connected *via* a carbon-carbon bond. Some examples of active compounds in this class are given in Figure 2.6. Pyrazofurin (Pyrazomycin) has a broad spectrum of antiviral activity as well as antitumour activity[45]. The compound is phosphorylated to the monophosphate and inhibits the decarboxylation of orotidine 5'-monophosphate (OMP) and uridine 5'-monophosphate (UMP)[46]. This accounts for its antitumour activity. Its antiviral properties may arise from inhibition of nucleic acid synthesis at a higher level.

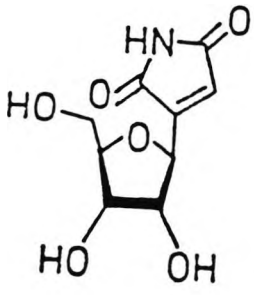
2.6 Figure C-Nucleoside Compounds.



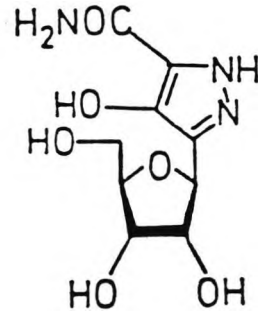
Pseudoisocystidine



Oxazinomycin



Shodomycin

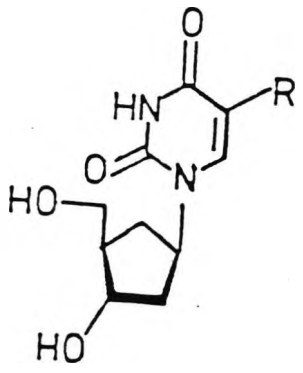


Pyrazomycin
(Pyrazofurin)

2.7.4 CARBOCYCLIC NUCLEOSIDE ANALOGUES[47-49].

A number of these compounds are also known in both the pyrimidine and purine series. The compounds have shown both antiviral and anti-cancer properties. Their modes of action are similar to the other nucleoside analogues. However, their medical applications are much less well documented.

Figure 2.7 Carbocyclic Nucleoside Class of Compounds.



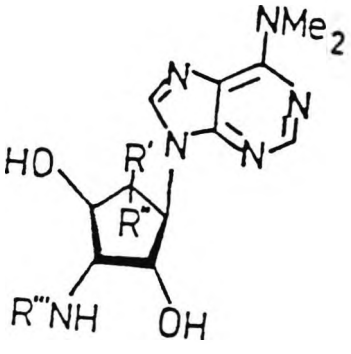
R =

Me

Br

I

NHMe



R' = OH, R'' = H

R' = H, R'' = OH

For both R' and R'':

R''' = $COCHCH_2C_6H_4$-p-OMe
|
NH_2

CHAPTER 3

GENERAL METHODS FOR THE SYNTHESIS OF 2′ -DEOXY,2′- SUBSTITUTED NUCLEOSIDES.

3.1 INTRODUCTION.

The synthesis of 2′-amino,2′ -deoxy,N^6 N^6-dimethyladenosine has been reported in poor yield[50]. The yields of intermediate compounds in the synthesis have been as low as 29%.In view of its biological significance, we set out to investigate synthetic routes in which a respectable yield of the compound was achievable. Our approach was to utilise the novel silicon-protecting reagent, 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2), recently available on the market and widely used for protecting the 2′-,3′-and 5′-hydroxyl functions of the sugar moiety of nucleoside molecules, in an attempt to achieve the above goal.In addition,the availability of the hypernucleophilic acylating agent, dimethylaminopyridine (DMAP) as a powerful tool in the acylation of hindered secondary alcohols has encouraged the possibility of higher yields and ease of synthesis.

Most nucleoside chemists appear to have used three distinctive approaches in their synthetic work. The approaches can be classified as (i)Transglycosylation reactions where the different functional groups on the sugar ring are manipulated before it is appended to the purine or pyrimidine bases to form the nucleoside (Figure 3.1,page 37).(ii)Intramolecular cyclisation reactions in which straight-chain molecules are cyclised to form either the sugar or the base part of the nucleoside mole-

cule (Figure 3.2,page 39) and (iii)Direct manipulation of the sugar and/or base functional groups of the nucleoside molecule either separately or concurrently (Figure 3.3,page 41). Comprehensive literature reviews of these methods of synthesis have been reported by White[51] (1981) and Mete[52] (1985).

3.2 TRANSGLYCOSYLATION REACTIONS.

The early approaches[53-55] to the synthesis of anomeric nucleosides of the furanose forms of 2/3-amino-2/3-deoxy-ribose have mostly utilised this method of synthesis. In most cases the starting point of the synthesis has been alpha-D-ribofuranoside in which the 2- or 3 -OH groups have been converted into protected amino functions. The other hydroxyl groups have been protected by different functional groups. In his approach, Wolfrom[56] (1966) converted the ribofuranoside into glycosyl chloride and then reacted the product with 6-acetamidomercuripurine to yield the 2' /3'-amino-2'/3'-deoxy adenosine. Hobbs and Eckstein[53] (1977) extended the synthetic procedure of Verheyden et al[54] (1971) to produce 2'-azido-2'-deoxyuridine in 50% yield.In this reaction the O(2),2'-cyclouridine was formed as an intermediate but it was not isolated. Eckstein's method for the synthesis of 2'-azido-2' -deoxyadenosine involved a typical transglycosylation reaction. He found it relatively easy to convert the sugar function of uridine into the 2'-azido sugar, which was then reacted with N^6-acetyladenine to produce both the alpha and beta isomers of 2'-azido-2'-deoxy adenosine, which were separated by column chromatography (Figure 3.1.a). Lee et al[55] (1970) used a similar method for the synthesis

Figure 3.1 Transglycosylation Reactions.

Figure 3.1.a[53]

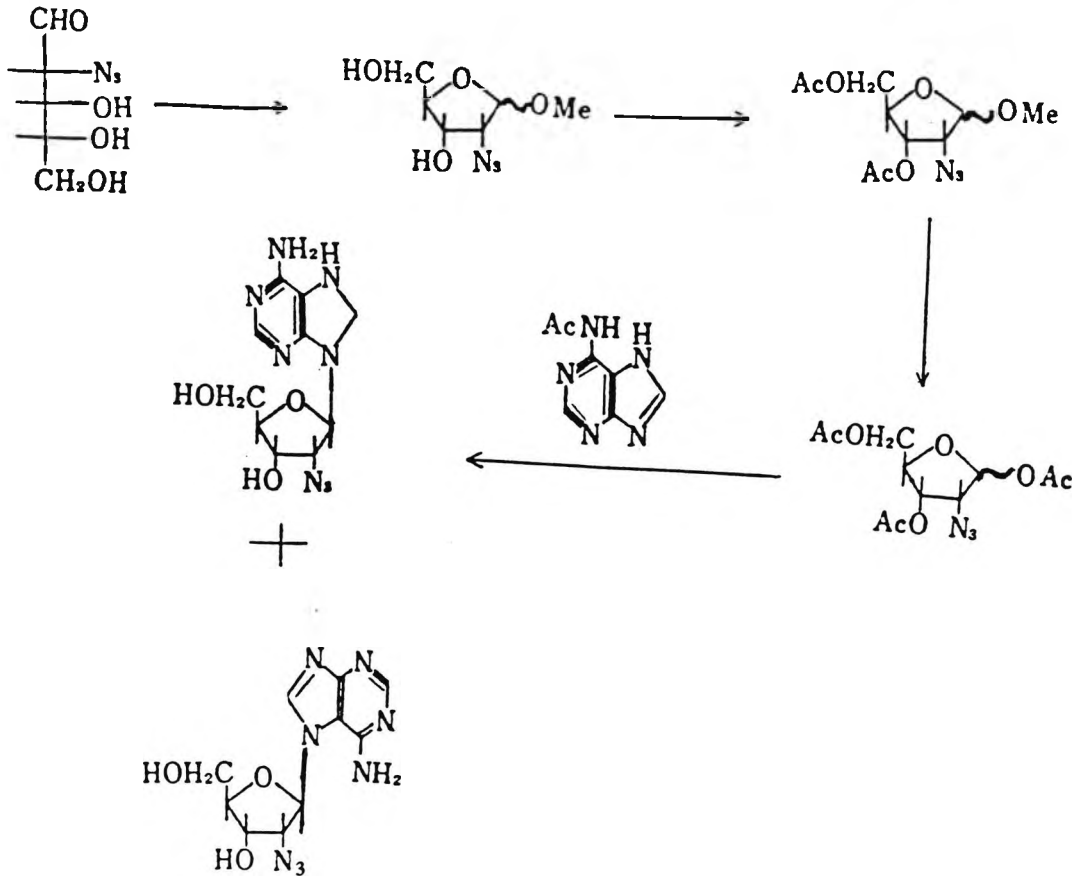
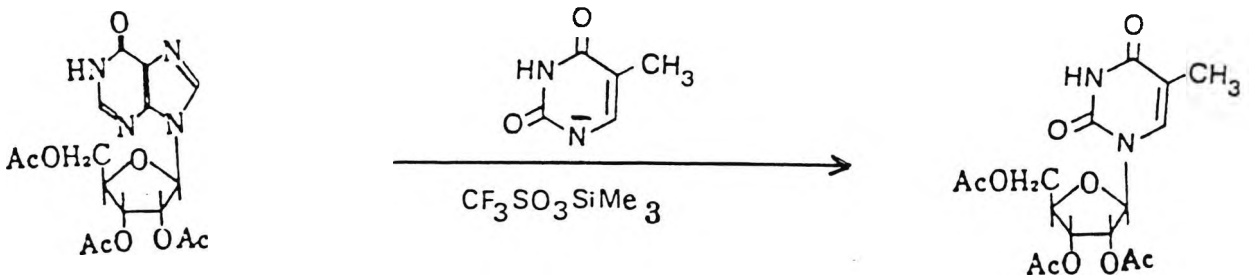


Figure 3.1.b[58]



of the naturally occurring antibiotic and antitumour drug Puromycin. They started initially by generating the protected ribofuranose moiety in which the 3-OH group was substituted by an amino group. Transglycosylation was effected by the reaction between the protected 3-aminoribofuranosyl sugar and 6-chloropurine in the presence of titanium tetrachloride. Similar transglycosylation reactions have also been reported by other workers[56,57] but using different hydroxyl-protecting groups. Recently transglycosylation reactions have been greatly improved by the use of trimethylsilyl triflates[58] (Figure 3.1.b).

3.3. INTRAMOLECULAR CYCLISATION REACTIONS.

Other workers[59-61] have started the synthesis of the nucleoside using the sugar molecule. Thus Buchanan[60] used D-mannono-1,4-lactone with the hydroxyl functions protected by acetyl groups, which was converted by cyclisation in two steps to the tri-acetyl mannopyrazole. In a series of subsequent steps and further cyclisation the pyrimidine ring was formed (Figure 3.2.a). The antibiotic araformycin (2'-Amino-3-arabino-furanosylpyrazalopyrimidine) was obtained. Similarly in the synthesis of Ara-A, Shaw[62] (1979) started with the sugar function, arabinofuranosyl chloride in which the hydroxyl groups were protected by benzyl groups. By reacting the latter compound with aminoimidazole carboxylic ester in hot acetonitrile in the presence of triethylamine, the protected arabinofuranosylimidazole carboxylate was obtained (Figure 3.2.b). This reaction has permitted the preparation of rela-

Figure 3.2 Intramolecular Cyclisation Reactions.

Figure 3.2.a[60]

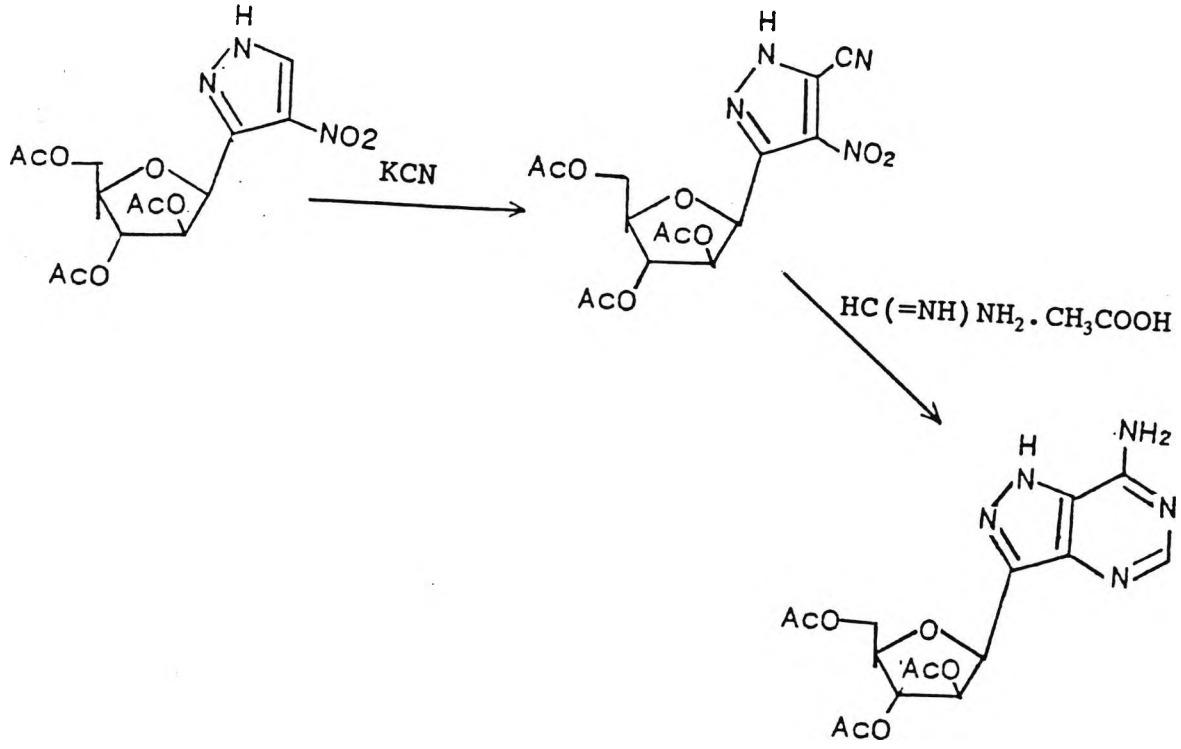
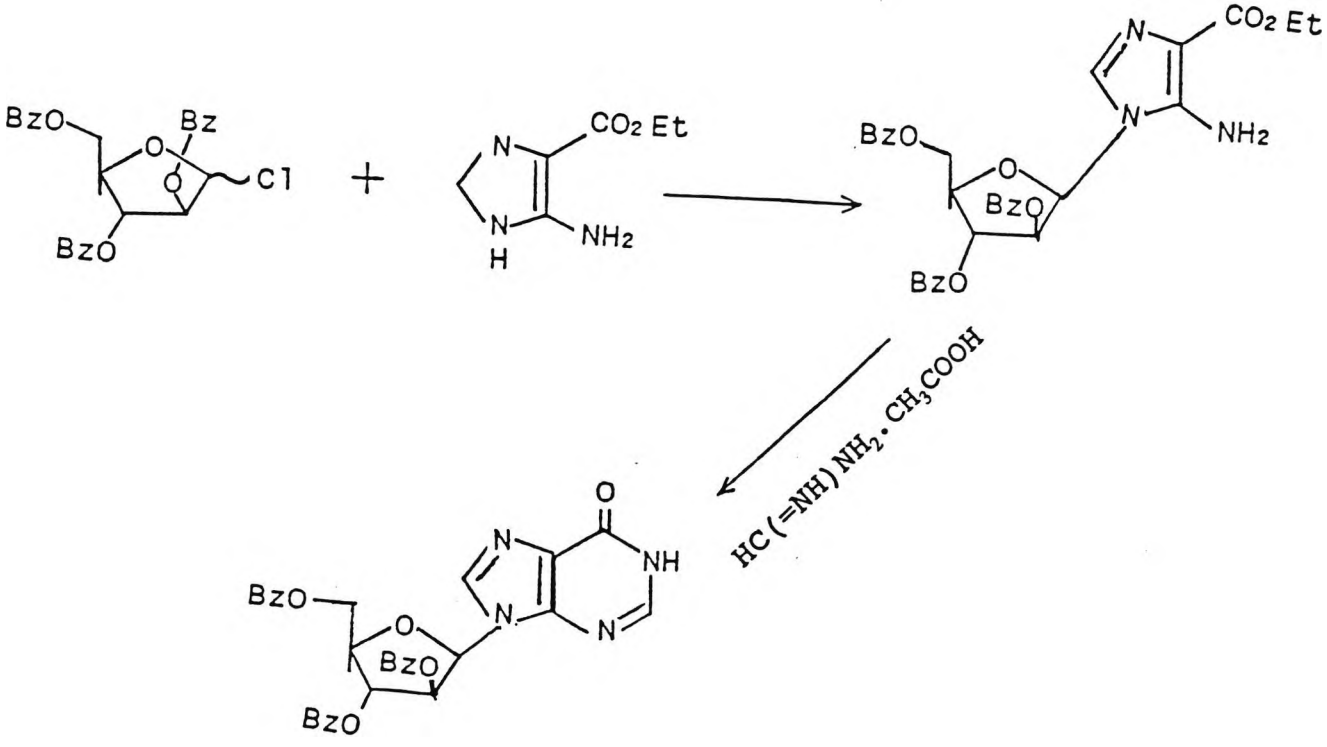


Figure 3.2.b[62]

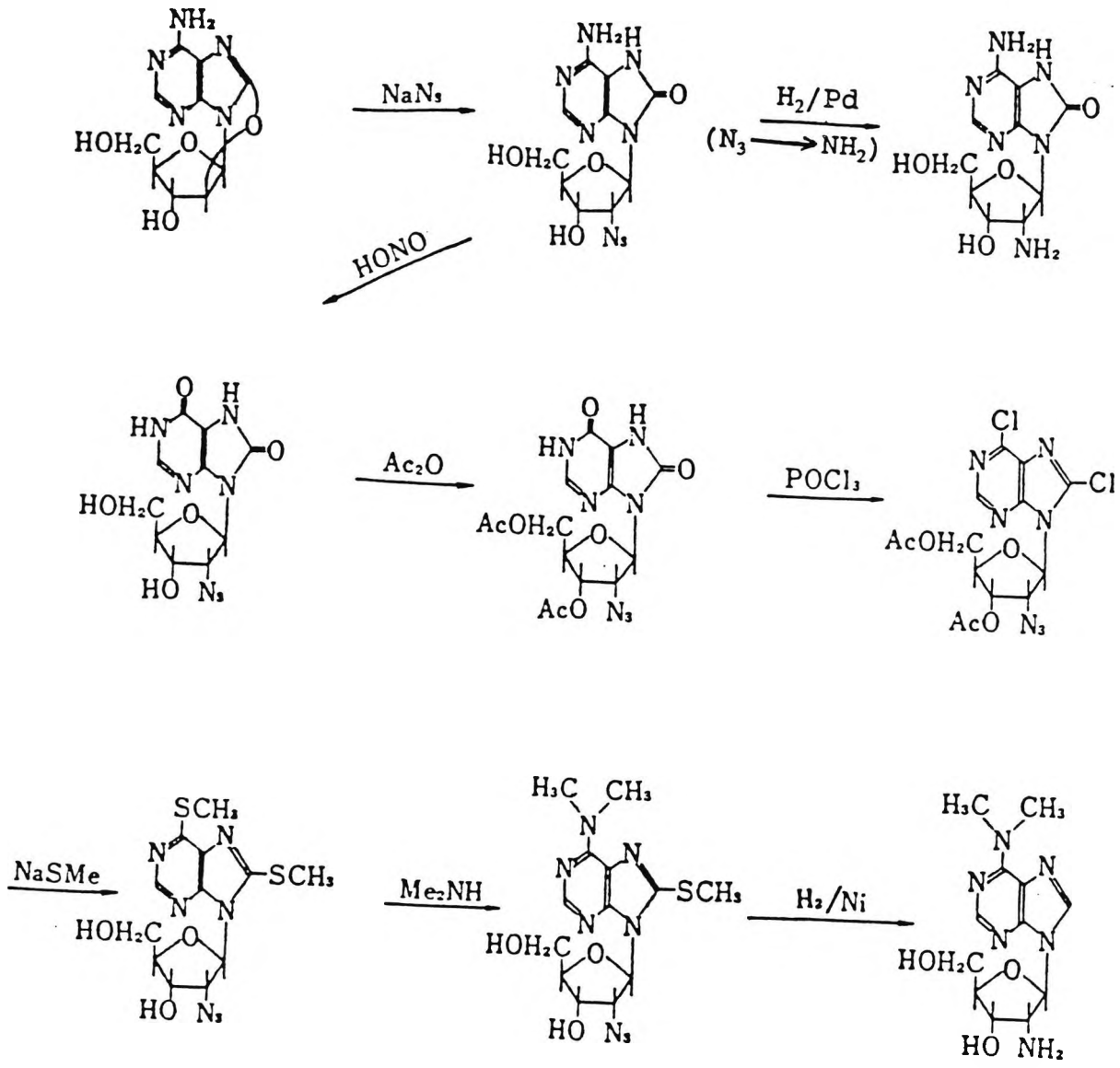


tively large amounts of aminoimidazole arabinoside in the desired beta-configuration without chromatographic separation. This compound was converted into Ara-inosine in one step by its reaction with formamidine acetate.

3.4 DIRECT MANIPULATION OF BASES AND/OR SUGAR FUNCTIONS.

With the commercial availability of suitable protecting compounds, this method of synthesis has acquired prominence, especially in the synthesis of 2′/3′-amino-2′/3′-deoxy-purines/pyrimidines. Ikehara's method of synthesis of 2′-amino-2′-deoxy nucleosides[50,63,64] involves direct manipulation of the functional groups on either the sugar or the base part in separate steps (Figure 3.3). The synthetic route starts initially with 2′,8-O-cycloadenosine, which is reacted with sodium azide to generate 2′-azido-2′-deoxy-8-oxyadenosine, thus introducing the 2′-azido group at an early stage (Figure 3.3). Two routes[50,64] were then selected: in the first, the azido compound was reduced using H_2/Pd to generate the 2′-amino, 2′-deoxyadenosine, while in the second route the amino function of the adenosine was deaminated by acetic acid/sodium nitrite to give the inosine derivative (Figure 3.3). Nucleophilic substitution by chlorine of the two oxy groups of the inosine compound afforded only 29% of the dichloro-substituted compound.In this sequence of reactions, the hydroxyl functions were protected using acetyl groups which are a commonly used protective function in nucleoside synthesis. A wide variety of other compounds have been used to protect reactive functional groups on the nucleoside molecule. Ikehara[65] and Reese[66] have

Figure 3.3 Direct Manipulation Of Sugar/Base[50,63,64].



used tosyl chloride, Robins[67] has used both trityl chloride (triphenylmethyl chloride) and tert-butyldimethylsilyl chloride and Binkley[68] has preferred the use of the isopropylidene group to protect hydroxyl functions on the sugar molecule. Reese[69] gives an exhaustive list of the methods used in hydroxyl protection in nucleotide synthesis. However, one recent widely used compound which has not been mentioned by Reese, but is important to the modern nucleoside chemists is well worth mentioning at this point.

The introduction by Markiewicz[70,71] of TIPDS-Cl_2 (1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane) set a precedent in the use of the compound by nucleoside chemists. It is used mostly for the selective protection of the 2′- and 3′- or the 3′- and 5′-hydroxyl functions of the sugar moiety of nucleosides. Its easy application, good stability in a large number of solvents and clean removal by fluoride ions, in most if not all cases by tetrabutyl ammonium fluoride (TABF), has made it popular as evidenced by its extensive use in nucleoside synthesis. Robins et al[72-74] have used the compound in the synthesis of the biologically-active ara-nucleosides which have strong anti-viral and anti-cancer properties. By the selective protection of the 3′- and 5′-hydroxyl functions, the 2′-OH group was first oxidised to a 2′-keto-nucleoside and then reduced to yield the predominantly ara-isomer. Similarly Van Boom[75] and Uedao et al[76] have used the compound in the synthesis of 2′-deoxy,2′-substituted nucleosides, namely the 2′-azido,2′-deoxy nucleosides, which on catalytic reduction give the 2′-amino,2′-deoxy-

nucleosides. The amino derivatives can be used as the starting compounds for the preparation of a whole host of 2′-substituted compounds.

TIPDS-Cl_2 is a bifunctional reagent which reacts rapidly with a primary hydroxyl group and then reacts intramolecularly with other suitably located primary or secondary hydroxyl groups. (Its reaction with tertiary alcohols has not so far been investigated). In the above-mentioned cases the second hydroxyl function is a secondary alcohol. This has shown the relative ease with which the 3′ -OH and 5′ -OH sites of most nucleosides can be protected. Robins et al[73] have shown that TIPDS can be used to protect the 2′-OH and 3′-OH positions of nucleosides simultaneously, although the reaction proceeded rather slowly.

In this study we have attempted two synthetic approaches. Originally we started with the direct manipulation of the nucleoside at both the sugar and the base functions independently. After we had encountered practical difficulties, we undertook the method of transglycosylisation. Once again we encountered further synthetic difficulties.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION.

The aim and purpose of this study is to formulate a viable route for the synthesis of the target compound, 2′[2-amino,3(p-methoxyphenyl)propanamido] 2′-deoxy,N^6N^6-dimethyladenosine - an isomer of Puromycin[77]. This compound would then be utilised as a precursor for the synthesis of compounds having varied functional groups attached to the amino function. In view of the high cost of using commercially available N^6N^6-dimethyl-adenosine as the starting material, a synthetic route for the large scale synthesis of 2′-amino,2′-deoxy,N^6N^6-dimethyladenosine was envisaged. The synthesis[50,64] of the latter compound is known, but the viability of the synthetic route in terms of intermediate product yields has given us cause for concern. In addition the availability of 1,3,dichloro-1,1,3,3-tetraiso-propyldisiloxane (TIPDS-Cl_2) which afforded relatively easy selective protection of the 3′-OH and 5′-OH groups of the sugar moiety, has rendered the 2′-position easier to functionalise. This aspect of the synthesis and the transglycosylation approach have been considered in this study.

Our synthetic route was set up as follows. Naturally occurring adenosine would be subjected to a series of chemical processes as described by two principal authors[50,63,66,76,78] to generate the biologically active compound 9-β-D-Arabinofuranosylade-nosine (Ara-A). The hydroxyl groups in the arabinose function of this compound would be protected using TIPDS-Cl_2, which

gives selective protection of the 3′- and 5′-hydroxyl groups. The unprotected 2′-position would be available for conversion into an azido derivative. Once this derivative was generated, the next step would be the manipulation of the purine molecule itself - the conversion of the 6-amino group into a 6-dimethylamino function. The route chosen was firstly the deamination of the 6-NH_2 group, to be followed by nucleophilic substitution of the site by a chlorine atom and finally by another nucleophilic substitution using a dimethylamino group. The azido group of the compound generated, 2′-azido,2′-deoxy,N^6N^6-dimethyl,3′,5′-di-O-TIPDS-adenosine would be reduced catalytically to give the amino function. Finally this amino group would be replaced with a p-methoxyphenyl-L-analyl group to generate the target molecule.

4.2 DIFFICULTIES ENCOUNTERED DURING SYNTHESIS.

Ikehara et al[63] (1975) has provided a convenient method for the large scale synthesis of the 2′,8-O-cyclonucleoside. In a further study Ikehara[50] (1977) converted the cyclonucleoside into 2′-azido, 2′-deoxy-8-oxyadenosine. After many steps and several intermediates, the compound 2′-amino,2′-deoxy,N^6N^6-dimethyladenosine was generated. This synthetic route has not proved to be very successful in terms of yields of intermediates and product. Other researchers[76] have also provided a route for the synthesis of 2′-amino,2′-deoxyadenosine, using TIPDS as a protective group for the hydroxyl functions of the sugar moiety. In our synthesis, the cyclonucleoside was converted into 8-hydrazino-ara-A[66,78]. The hydrazino function

was subsequently removed by alkaline hydrolysis. This route for the synthesis of the commercially expensive Ara-A, though lengthy is simple and clean and does not involve any separation procedures such as chromatography.The crude intermediates generated along the synthetic route were pure enough to be utilised as starting materials for the next reaction. The reaction of Ara-A with TIPDS-Cl_2 provided the desired protected 3',5'-compound as the only product.

The substitution of the 2'-OH group by the azido function via the triflate reaction and the removal of TIPDS by tetrabutyl ammonium fluoride (TBAF) to generate 2'-azido,2'-deoxyadenosine provided no major difficulties. Several attempts were made to deaminate the amino function of the TIPDS-protected 2'-azido, 2'-deoxyadenosine, using acetic acid and sodium or isopropyl nitrite. Follow-up of the reactions after several hours by TLC gave no products. However, deamination did take place with acetic acid and sodium nitrite on the unprotected compound to generate the inosine derivative.

Similarly, 3',5'-di-O-TIPDS inosine would not undergo chlorination with phosphorus oxychloride; while on the other hand 2', 3',5'-triacetoinosine readily underwent chlorination at the 6-position without any side products[79-81]. When 6-chloroinosine triacetate was treated with a very large excess of dimethylamine, N^6N^6-dimethyladenosine was obtained in quantitative yield. When the dimethyladenosine was reacted with TIPDS-Cl_2, the 2',3'-di-O-TIPDS derivative was obtained after a prolonged

reaction time, instead of the expected 3',5' -di-O-TIPDS compound. Robins et al[72] have synthesised the TIPDS-derivatives of a number of nucleosides for the purpose of conformational studies, but the synthesis of dimethyl-TIPDS-adenosine has not been reported previously.

Robins et al[67,82] have also described the synthesis of TIPDS-ara-A via the oxidation and reduction of the 2'-OH function of TIPDS-adenosine. Adenosine was reacted with TIPDS-Cl_2 to produce 3',5'-di-O-TIPDS-adenosine, which was then oxidised at the 2'-position. A 1:2:1 complex of CrO_3/pyridine/acetic anhydride (Garregg-Samuelson reagent[83]) was used in the oxidative process to generate the 2'-keto derivative. On reduction of this compound with sodium borohydride, a mixture of the two epimers was obtained, which required an extensive purification process. Even under rigorous conditions of purification, chromium metal contamination still persisted. The product yield was greatly reduced as a result of the refining process.

With the relatively easy synthesis and improved yield of dimethyladenosine, we contemplated the route of transglycosylation as a method for the synthesis of 2'-amino,2'-deoxy,N^6N^6-dimethyladenosine. Fleet et al[84] (1986) have reported the conversion of ribonolactone to 2-azido-1,4-ribonolactone. We have prepared the latter compound in good yield. Catalytic hydrogenation of the 2-azidolactone gave a product which had lost all the features of the starting material. Also attempts to protect the 3- and 5-hydroxyl functions of the azidolactone

using TIPDS-Cl_2 gave a product which did not have the properties of the starting compound. Previously Fleet[84] (1986) and Scriven and Turnbull[85] (1988) had attempted to catalytically reduce the 2-azido-lactone for the synthesis of 2-amino-sugars. Their product was identical (spectroscopic analysis and melting point) to the one which we produced. Fleet helped us to establish the chemical structure of this novel compound as 3,4-dihydroxyproline by NMR analysis (see Experimental, Section 6.4.6).

The cost of materials was an important consideration in the present study. TIPDS-Cl_2 was commercially available with 90% purity, but in view of the very high cost of the compound and the large quantity which was required for our purpose, we undertook its synthesis[86], devising our own improved method as described in Scheme V, page 64.

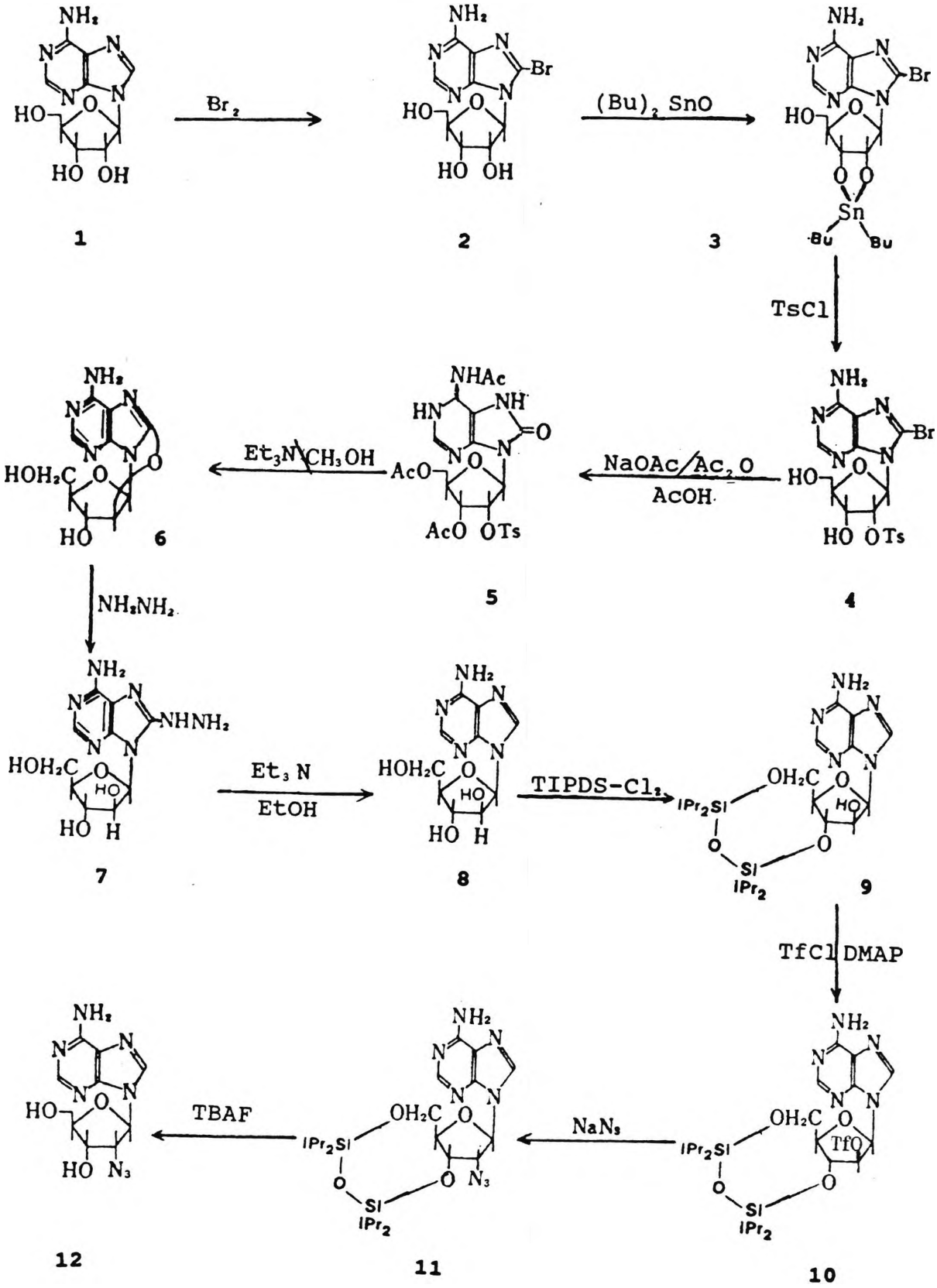
CHAPTER 5

METHODS OF SYNTHESIS.

5.1 SCHEME I - Synthesis Of The Target Compound Via The Synthesis Of Ara-Adenosine.

For the synthesis of 2'-azido,2'-deoxy adenosine, it was thought that the best starting point would be 9-β-D-arabinofuranosyl-adenosine (Ara-A). Naturally occurring adenosine was converted to Ara-A by a series of procedures used by Ikehara et al[63,65,87] to synthesise 2',8-cycloadenosine in high yield. The latter compound can be easily converted to Ara-A via the hydrazino intermediate[76,78]. This method is suitable for the large scale synthesis of 2',8-cycloadenosine and thus Ara-A. This 7-stage procedure (Scheme I) was selected on the merit that it did not involve any laborious or sophisticated steps and the yield of product with each step was high.

Adenosine was shown to undergo electrophilic substitution by Kruger[88] as early as 1892. He showed the ease with which the purine ribosides underwent bromination and chlorination. With an improved method, we have brominated adenosine in 94% yield. When naturally occurring adenosine (Compound 1) was reacted with bromine water in an acidic medium, 8-bromo-adenosine (Compound 2) was obtained in quantitative yield. Complete bromination was confirmed by ^1H NMR analysis on the crude product, which showed the absence of a signal for the C-8 proton at δ8.20. Debromination and nucleophilic substitution occurred relatively easily under mild basic conditions as shown


Br2
(Bu)2 SnO
TsCl
NaOAc/Ac2O
AcOH
Et3N/CH3OH
NH2NH2
Et3N
EtOH
TIPDS-Cl2
TfCl DMAP
NaN3
TBAF
1
2
3
4
5
6
7
8
9
10
11
12

SCHEME I

by the treatment of 8-bromoadenosine with tosyl chloride in the presence of triethylamine.

For the selective tosylation of the 2′-position,8-bromoadenosine was first converted to 2′,3′ -di-n-butyltin-8-bromoadenosine (Compound 3) as described by Wagner et al[89]. Such a procedure ensures selective tosylation.8-Bromoadenosine was heated under reflux with a molar equivalent of di-n-butyltin oxide in anhydrous methanol for 5 hours.The product, 2′,3′-di-n-butyltin-8-bromoadenosine was filtered off on cooling as a white crystalline compound. The instability of the compound was shown when it reverted to 8-bromoadenosine on TLC analysis. Tosylation at the 2′-position was carried out by reacting the tin derivative of 8-bromoadenosine with a five-fold excess of tosyl chloride at ambient temperature to yield 2′-O-tosyl-8-bromoadenosine (Compound 4).

The method used for the acetylation of 2′-O-tosyl-8-bromo-adenosine[64,65] (Compound 4) is the one commonly used for most nucleosides. This method for the protection of the hydroxyl groups of the sugar function has been widely reported. It has been shown that the acetyl groups can be easily inserted and removed using common bench reagents. In most cases, the nucleoside compound is refluxed with acetic anhydride and glacial acetic acid in the presence of anhydrous sodium acetate. Complete acetylation and nucleophilic substitution at the C-8 position were effected by heating compound 4 under reflux with acetic anhydride and glacial acetic acid in the

presence of anhydrous sodium acetate for 3 hours. The highly crystalline triacetate derivative of 2′-O-tosyl-8-oxyadenosine (Compound 5) was obtained. An added advantage of using the acetyl group as a protecting group is the ease with which it can be removed by mild alkaline conditions. Deacetylation and cyclisation were effected when the triacetyl derivative of 2′-O-tosyl-8-oxyadenosine (Compound 5) was heated under gentle reflux with a large excess of triethylamine in dry methanol over 60 hours. 2′,8-O-Cycloadenosine (Compound 6) was the only product generated[64].

When 2′-8-O-cycloadenosine (Compound 6) was heated under reflux with a large excess of hydrazine in absolute ethanol, 8-hydrazino-ara-A (Compound 7) was obtained in over 80% yield[66,78]. Removal of the hydrazino group by heating compound 7 under reflux with dry triethylamine in anhydrous ethanol over 16 hours gave the biologically active compound 9-β-D-arabinofuranosyladenosine (Ara-A) (Compound 8) in quantitative yield.

The synthesis of most TIPDS-protected nucleosides involves more or less similar methods of reaction and work-up.Two approaches were adopted for the synthesis of 3′,5′-di-O-TIPDS-ara-adenosine[72-74] (Compound 9). In Scheme I, Ara-A was stirred with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2) in dry pyridine at ambient temperature in an inert, anhydrous atmosphere to generate the protected Ara-A[70]. The product was obtained in quantitative yield. For the other method of synthesis of 3′,5′-di-O-TIPDS-ara-A (Scheme II), the starting

material was adenosine.

Before the synthesis of 2′-azido-2′-deoxyadenosine (compound 12) could be effected, the substitution of the 2′-OH group by a good leaving group was carried out. One functional group which was shown to be quite versatile for our purpose, was the triflate group, inserted effectively in the presence of the hypernucleophilic agent dimethylaminopyridine[73](DMAP). When the protected Ara-A was reacted with trifluoromethanesulphonyl chloride in the presence of DMAP in dry dichloromethane at 0°C, the 2′-trifluoro-protected Ara-A derivative (Compound 10) was obtained as the only product. When the latter compound was reacted with sodium azide, nucleophilic substitution by the azido group gave 2′-azido-3′,5′-di-O-TIPDS-adenosine (Compound 11). Removal of TIPDS was then carried out by the reaction of compound 11 with tetrabutylammonium fluoride (TBAF) to give 2′-azido-2′-deoxyadenosine (Compound 12).

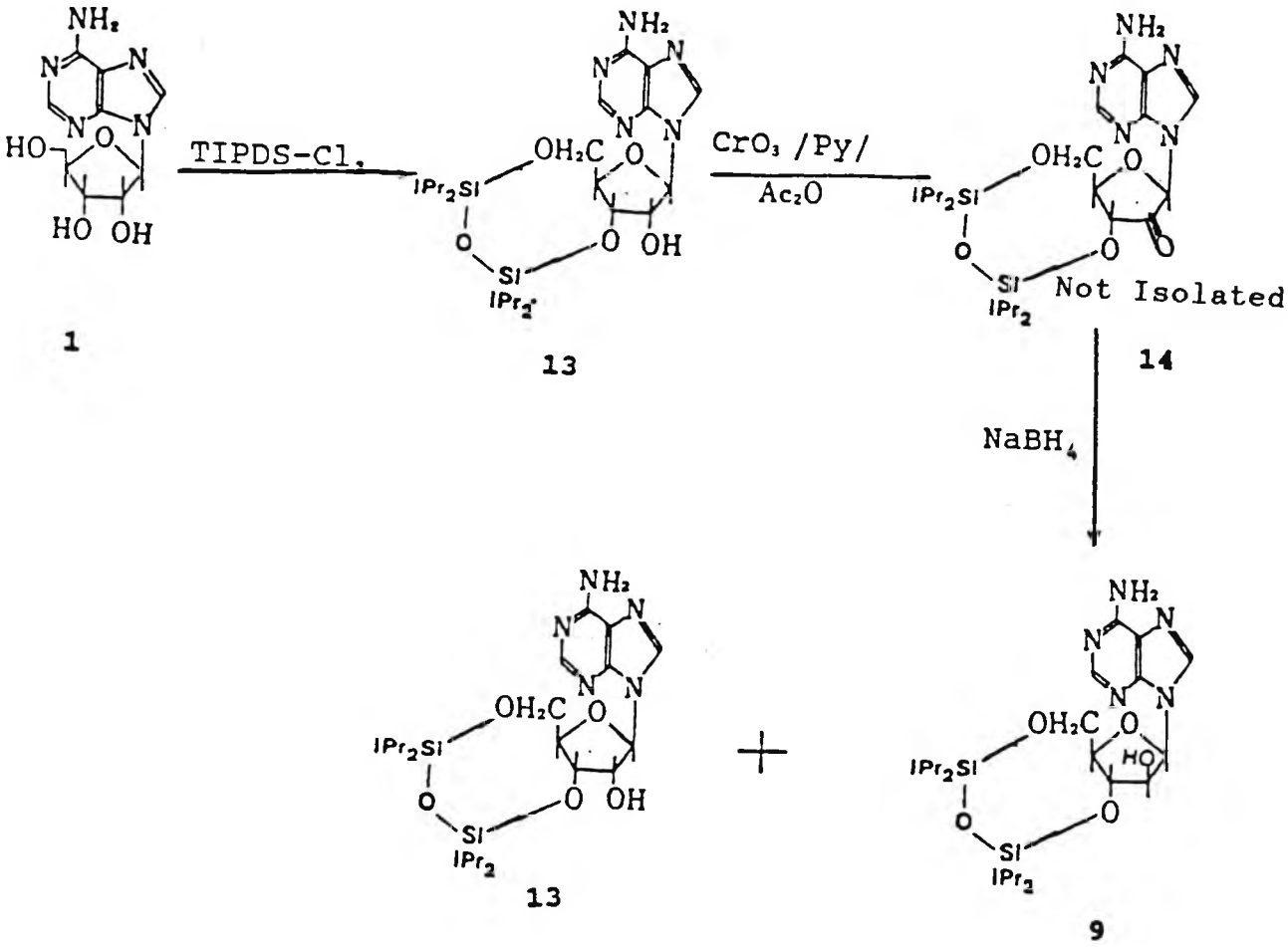
In the subsequent stages, the synthesis of the target compound, 2′-azido,2′-deoxy,N^6 N^6-dimethylaminoadenosine was envisaged.The starting material was Compound 11. But none of the standard reactions that were planned took place. It was thought that by using the standard method of deamination in which the 6-amino function of Compound 11 was reacted using either sodium or isopropyl nitrite in acetic acid, the inosine derivative would be generated. Then by a nucleophilic substitution reaction,the 6-oxy-atom would be replaced by chlorine to generate the 6-chloro-compound, which when reacted with dimethylamine and

deprotected with tetrabutyl ammonium fluoride would have generated the target compound, 2′-azido,2′-deoxy,N^6 N^6-dimethyl adenosine. We could not deaminate compound 11 by running several reactions under a variety of conditions. We attempted to investigate the reasons for our failure by using inosine as the starting compound (Scheme III).

5.2 SCHEME II - Synthesis Of The Target Compound Via Oxidative-Reductive Processes.

Adenosine (Compound 1) was converted to 3′,5′-di-O-TIPDS-adenosine[47-49] (Compound 13) in an identical manner to that used for its Ara-A epimer (Compound 8: Scheme I). Once the 3′- and 5′-hydroxyl groups had been protected, it was relatively easy to substitute the 2′-OH group by other functional groups. The selective oxidation of the 2′-OH group was carried out as described by Robins et al[67,82], using a 3 molar equivalent of a premix complex of CrO_3/pyridine/Ac_2O in dichloromethane at 0°C and allowing the mixture to return to room temperature and stirring it at this temperature over 45 minutes. The complex was dissolved in ethyl acetate and filtered using silica to remove all undissolved particles. The solvent was evaporated off under vacuum while maintaining a temperature of below 25°C. The 2′-keto compound (Compound 14: SchemeII) was not isolated because of its instability and the reduction reaction was carried out *in situ* with sodium borohydride dissolved in a minimal amount of water. The route to the generation of 3′,5′-di-O-TIPDS-ara-A in this scheme can be looked at as a "one pot reaction". TLC analysis of the product showed the presence of two compounds (Compounds 9 and 13), which were separated by column chromatography. Compound 9 was the predominating compound and it was eluted first. Compound 13, the starting material which was unchanged, was subjected to further oxidation and separation. For conversion of compound 9 to the triflate derivative and subsequently to the azido group, the methods described in scheme I were used. Deprotection was also

carried out in a similar manner to that described previously.



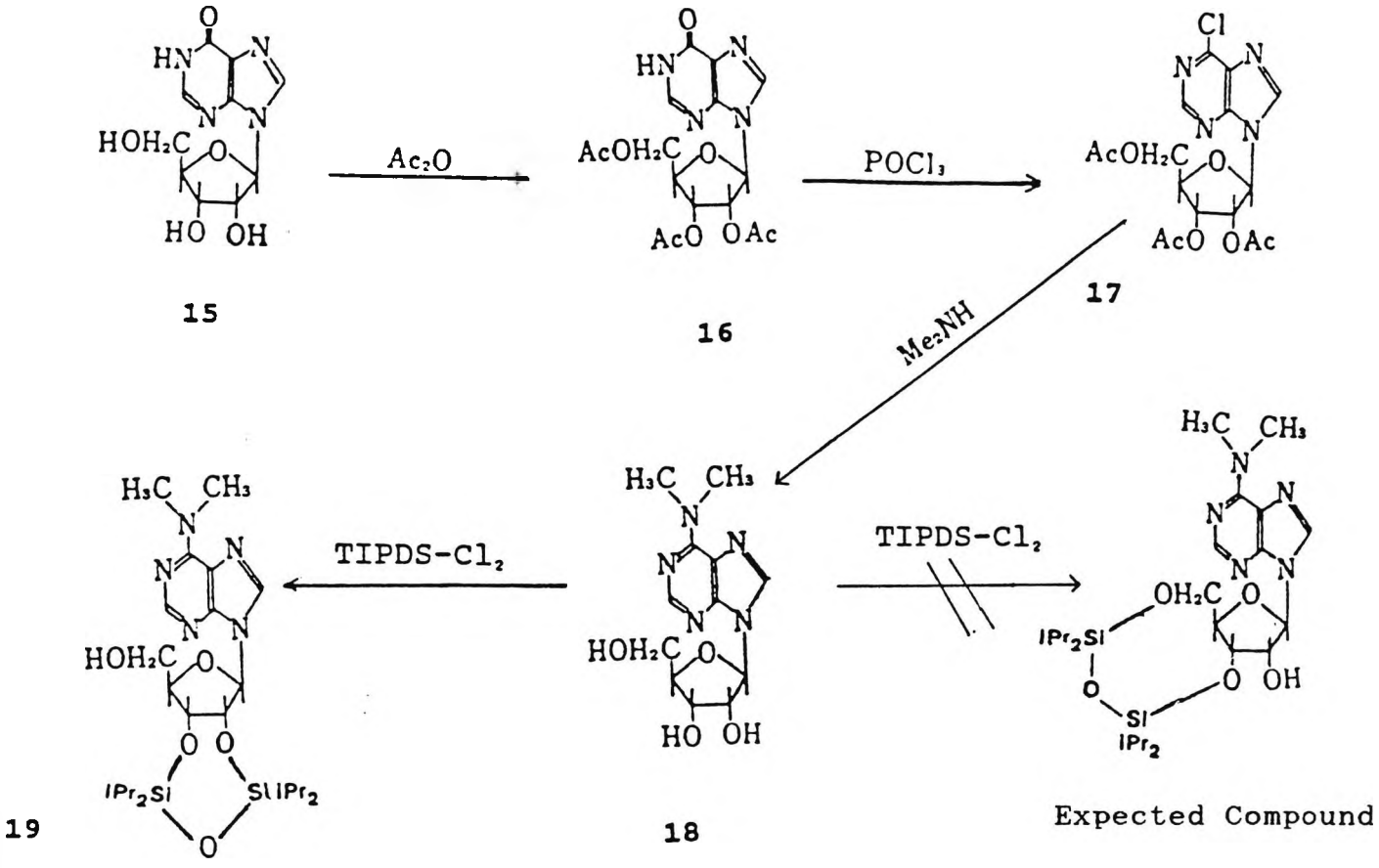
SCHEME II

5.3 SCHEME III - Synthesis Of The Target Compound From Inosine.

For another attempted route to the synthesis of N^6N^6-dimethyl, 2′-azido-2′-deoxyadenosine, naturally occurring inosine (Compound 15) was used. The hydroxyl functions of the sugar moiety of inosine were first protected by acetylation[63] to give the highly crystalline compound inosine-2′,3′,5′-triacetate (Compound 16). The standard method of nucleoside acetylation and reaction workup as described in Scheme I were used. The next stage involved the chlorination of C-6. Substitution of the 6-oxy group was carried out by refluxing triaceto-inosine (Compound 16) with a 5 molar equivalent of phosphorus oxychloride and a 2.5 molar equivalent of DMAP in anhydrous acetonitrile[80,81]. The reaction was followed by TLC and at completion, one compound, namely the 6-chloro derivative (Compound 17) was isolated. Attempts to recrystallise the compound gave side-products as shown by TLC.

Reaction of the 6-chloro compound with excess dimethylamine at ambient temperature in ethanol yielded N^6N^6-dimethyladenosine (Compound 18) in quantitative yield. The subsequent step was, firstly the protection of the 3′- and 5′-hydroxyl groups of the ribose molecule using TIPDS-Cl_2 and secondly the functionalisation of the 2′-position. In a prolonged reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, N^6N^6-dimethyladenosine (Compound 18) gave the unexpected 2′,3′-di-O-TIPDS-dimethyl adenosine (Compound 19) instead of the expected 3′,5′-di-O-protected compound.

In a different approach, inosine (Compound 15) was reacted with 1,3-dichloro-TIPDS under similar conditions to those used for other nucleosides (See Schemes I and II) and the expected 3 ,5-di-O-TIPDS-inosine was obtained, as confirmed by NMR spectroscopy. The $2'$ -OH group was then protected with an acetyl group using the standard acetylating reagent and reaction conditions. An attempt was made to chlorinate the product using the procedure described previously but substitution at C-6 did not occur, as would be expected under the conditions used with inosine triacetate (Compound 16, Scheme III).



15 16 17 18 19

Unexpected Compound

Expected Compound

SCHEME III

5.4. SCHEME IV - Synthesis Of The Target Compound Via The Transglycosylation Method.

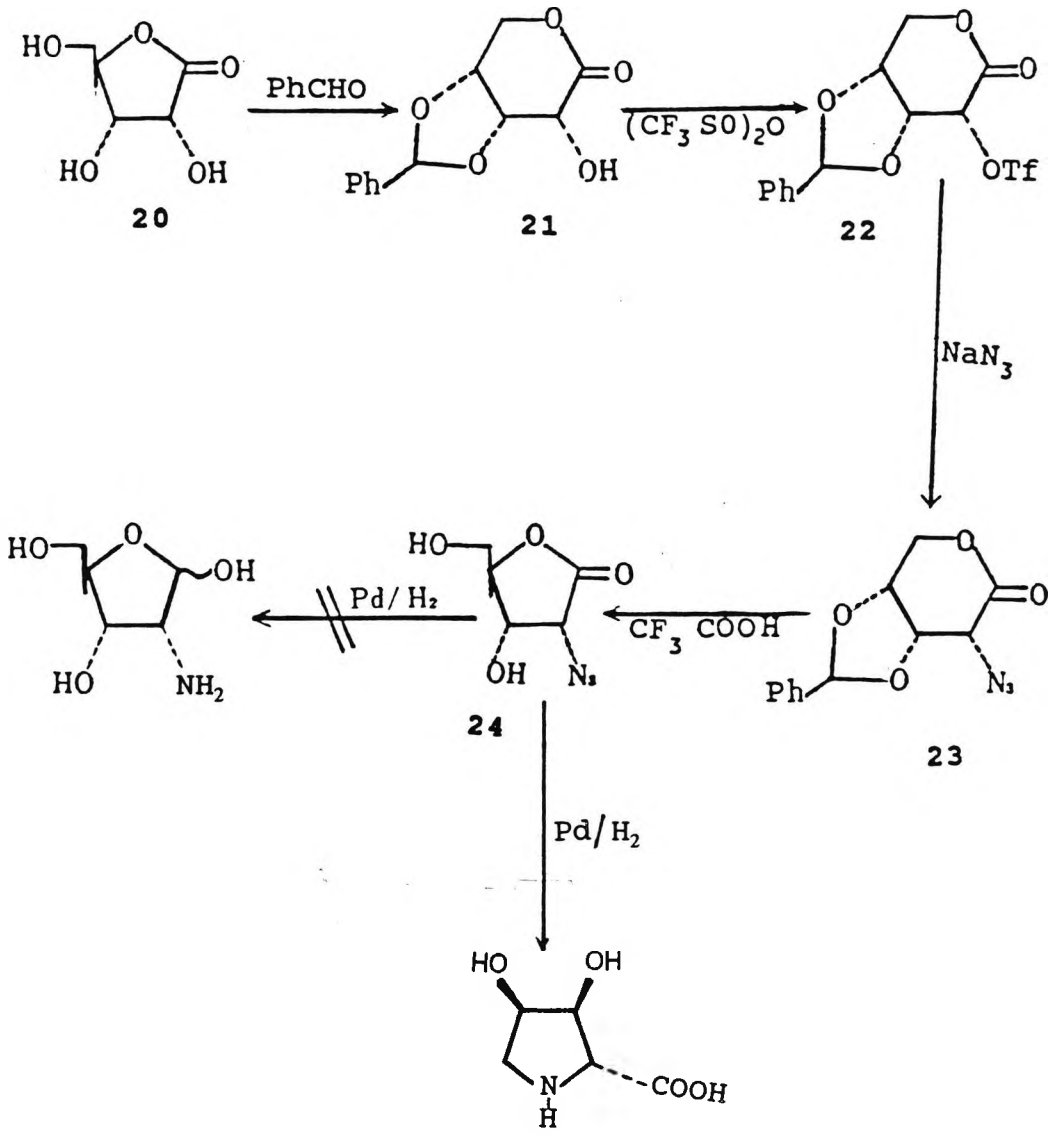
In this scheme we envisaged the preparation of 2-amino-2-deoxyribose for the synthesis of 2 -amino-2 -deoxy-N^6,N^6-dimethyladenosine using the method of transglycosylation as described by Hobbs and Eckstein[53]. The starting material used was D-ribono-1,4-lactone (Compound 20). When the latter compound was reacted with benzaldehyde in concentrated hydrochloric acid and shaken over 3 hours at room temperature, 3,4-benzylidene-D-ribono-1,5-lactone (compound 21) was obtained in quantitative yield. This reaction was used as a method for the protection of the 3- and 4-hydroxyl functions of the lactone, thus leaving the 2-position open for further functionalisation. To convert the C-2 position into a better nucleophilic centre compound 21 was converted to the triflate derivative (Compound 22).

The reaction conditions were similar to those described in Schemes I and II but without the presence of DMAP. Compound 21 was suspended in dry pyridine in an inert atmosphere with the mixture cooled to between -10 and -20°C. Trifluoromethanesulphonic anhydride was added dropwise until the mixture was homogeneous. The product was extracted with ethyl acetate and on evaporation of the solvent, the triflate derivative (Compound 22) was obtained. The crude triflate compound was then treated with sodium azide in DMF for 1.5 hours. The protected 2-azido derivative (Compound 23) was isolated when the reaction mixture was poured into ice water and extracted

with ethyl acetate. On evaporation of the solvent the azido derivative (Compound 23) was obtained.

Deprotection of Compound 23 with a strong organic acid afforded the compound 2-azido-2-deoxylactone (Compound 24). The protected 2-azido derivative (Compound 23) was dissolved in aqueous trifluoroacetic acid and stirred at 50°C for 1.5 hour. The product (Compound 24) was separated by flash chromatography.

The 2-azido lactone was then catalytically reduced with hydrogen in the presence of palladium with view to the generation of 2-amino-2-deoxyribose. Instead the unexpected compound 3,4-dihydroxyproline[84,85] was obtained.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OH
21
$(CF_3SO)_2O$
O
O
O
Ph
OTf
22
NaN_3
O
O
O
Ph
N_3
23
CF_3COOH
HO
O
O
OH
N_3
24
Pd/H₂
HO
O
OH
HO
NH_2
Pd/H_2
HO
OH
N
H
COOH

SCHEME IV

5.5 SCHEME V - Synthesis Of 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2).

This scheme describes the synthesis of the reagent 1,3,dichloro-1,1,3,3-tetraisopropyldisiloxane[86] (Compound 28). The starting point of the synthesis is the Grignard reagent, isopropylmagnesium chloride (Compound 25). As with all Grignard syntheses, rigorous anhydrous conditions have to be observed.Oven-dried magnesium turnings were placed in a round-bottom flask and previously dried (sodium wire) diethyl ether was added. Isopropyl chloride was added dropwise to maintain control of the reaction. Once the addition was completed, the mixture was stirred at ambient temperature and then refluxed overnight. The Grignard reagent, isopropylmagnesium chloride (Compound 25) was obtained and stored in diethyl ether.

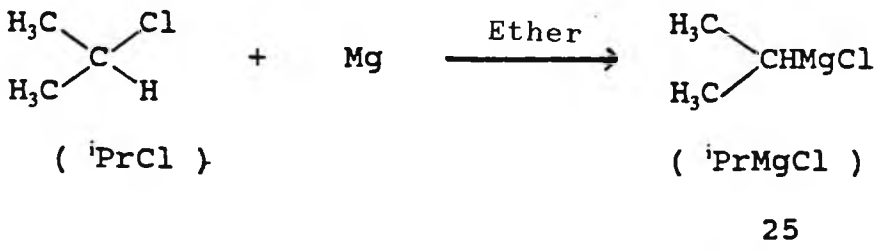
The reaction between isopropylmagnesium chloride and trichlorosilane ($SiHCl_3$) is violent and explosive and proper temperature control together with controlled addition of the reagent is critical. A temperature of below $-20^{o}C$ was maintained during the addition reaction. After the addition was completed, the mixture was stirred and allowed to return to room temperature. For acid hydrolysis similar temperature and addition controls were required. After hydrolysis, the organic layer was separated and extracted to leave diisopropylsilanol as an oil (Compound 26), which was stored under nitrogen.

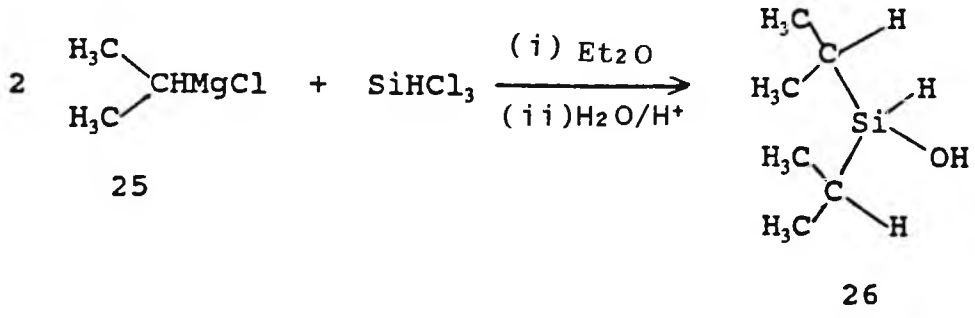
Dehydration of diisopropylsilanol with phosphorus pentoxide and fractionation under reduced pressure in a nitrogen atmosphere

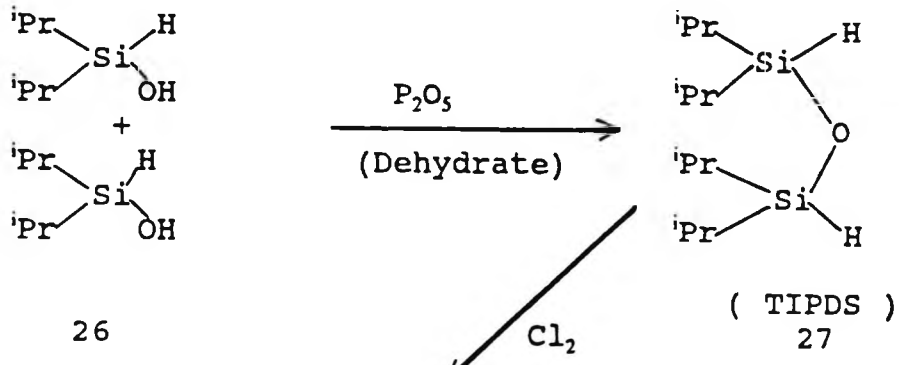
gave 1,1,3,3-tetraisopropyldisiloxane (Compound 27) as an oil. This compound was also stored under nitrogen. Its IR spectrum showed the presence of a strong Si-H band at 2150cm^{-1}.

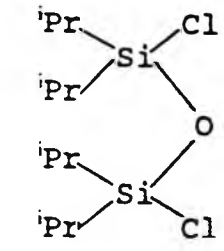
Compound 27 was dissolved in anhydrous carbon tetrachloride in an inert atmosphere and the solution was cooled to 0°C. Chlorine gas was bubbled into the solution until it was persistently yellow. IR analysis confirmed the formation of the Si-Cl bond as shown by the complete absence of the Si-H band at 2150cm^{-1}. On evaporation of the carbon tetrachloride under nitrogen and under reduced pressure, an oil was obtained. The oil was fractionated under reduced pressure to yield 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (Compound 28) in high yield. The compound was stored in an anhydrous nitrogen atmosphere.

Preparation Of 1,3-Dichloro,1,1,3,3-Tetraisopropyldisiloxane.









SCHEME V

CHAPTER 6

EXPERIMENTAL SECTION

TABLE OF CHEMICAL REAGENTS USED.

A. SUPPLIERS - ALDRICH CHEMICAL COMPANY.

	% PURITY
Acetic acid	99.7
Acetic anhydride	99+
Adenosine	98
Bromine	99.99
Benzaldehyde	99
Chromium(VI) trioxide	99
Dibutyltin oxide	98%
4-Dimethylamino pyridine	99
Dimethylamine in water	40
Hydrazine	98
Hydrochloric acid	
Inosine	99
Isopropyl chloride	99
Magnesium sulphate - anhydrous	99
Magnesium turnings	98
Palladium black	
Phosphorus oxychloride	99
Phosphorus pentoxide	98+
Sodium acetate - anhydrous	99
Sodium azide	99
Sodium bicarbonate	99.7
Sodium borohydride	98

Sodium chloride	99+
Sodium nitrite	97+
Tetrabutylammonium fluoride	99
p-Toluenesulphonyl chloride	99
Trichlorosilane	99
Triethylamine	99
Trifluoroacetic acid	99+
Trifluoromethanesulphonic anhydride (stored under N_2)	99
Trifluoromethanesulphonyl chloride (stored under N_2)	99

SIGMA LABORATORY

Isopropyl nitrite	97+
1,4-Ribonolactone	98

GASES - (Supplied by University suppliers)

Nitrogen - (Dried over conc. sulphuric acid)

Hydrogen - (Dried over conc. sulphuric acid)

Chlorine - (Supplied by Aldrich Chem. Co. Ltd)	99.7

B.PURIFICATION OF COMMERCIALLY AVAILABLE REAGENTS USED.

1. Dried with anhydrous $MgSO_4$ then distilled.

Methyl alcohol

Ethyl alcohol

2. Dried with P_2O_5 then distilled and stored over molecular sieve.

Carbon tetrachloride

Chloroform

Dichloromethane

3. Distilled and stored over molecular sieve.

Dimethylformamide

Pyridine

Tetrahydrofuran

4. Dried with CaH_2 and distilled.

Diethyl ether (stored over sodium wire)

Ethyl actate

Acetonitrile

Petroleum ether 60 - 80°C.

5. Stored under N_2. (Prepared reagent).

1,3-Dichloro,1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2).

C.ANALYTICAL INSTRUMENTS USED.

1. INFRARED SPECTROPHOTOMETER.

   Perkin-Elmer 983G.

2. ULTRA VIOLET SPECTROPHOTOMETER.

   Perkin-Elmer 402.

3. NUCLEAR MAGNETIC RESONANCE (NMR).

   (i) JEOL FX60 60MHz ^{13}C nmr.

   (ii) JEOL 100MHz ^{1}H nmr.

4. MASS SPECTROPHOTOMETER.

   MS 30.

EXPERIMENTAL.

SCHEME I

6.1.1 8-Bromoadenosine[87] (2).

Adenosine (21g,0.08 mol) was dissolved in a sodium acetate buffer (0.5M,pH4,400ml) with continuous stirring and slight warming. The resultant clear solution was allowed to return to room temperature and bromine (0.08 mol, 4.1ml) was added. The solution was stirred at room temperature for 3 hours and the colour of the solution was discharged with sodium meta-bisulphite. The pH of the solution was adjusted to 7 with 5M NaOH and then left overnight in a refrigerator. The pale yellow crystalline precipitate formed was filtered off, washed successively with water (50ml) and acetone (50ml) and dried over magnesium sulphate (Yield 21.96g). The mother liquor was evaporated to half its volume and a second crop of 8-bromo-adenosine was obtained (1.84g). (Total yield 23.80g, 94%). For characterisation, a sample was recrystallised from water; m.pt 201°C with decomposition (Lit[87] 200°C). (Found: C,34.82; H, 3.54; N, 20.01. $C_{10}H_{12}N_5O_4Br$ requires C, 34.69; H, 3.49; N, 20.23).N.M.R analysis showed the absence of a signal for the C-8 proton at δ 8.2, confirming bromination at C-8.

6.1.2 2′,3′-Di-n-butyltin-8-bromoadenosine[63,65] (3).

8-Bromoadenosine (Compound 2) (4.152g, 12mmol) and di-n-butyl-tin oxide (3.0g, 12mmol) were dissolved in anhydrous MeOH (300ml) and the mixture was heated under reflux for 5.5hr.

Heating was stopped and the flask was allowed to return to room temperature. Pale pink crystals precipitated on cooling. Recrystallisation from anhydrous MeOH yielded a white crystalline product. (Yield 6.28g, 11mmol, 92%); m.pt 221-222°C (Lit[63] 220-221°C). This compound reverted to the starting material on TLC ($CHCl_3$:MeOH eluting solvent). (Found: C,37.53; H, 4.88, N, 11.93, Br, 14.02.$C_{18}H_{28}N_5O_4BrSn$ requires C, 37.47; H, 4.89; N, 12.14; Br 13.85). UV,λ_{max} (EtOH) 266nm. N.M.R (d_6-DMSO) 7.97 (s, 1H, H-2), 7.53 (s, 2H, 6-NH_2), 5.87 (d, 1H, H-1′), 5.55 (q, 1H, H-2′), 4.21 (br, 1H, H-3′), 3.85 (br, 1H, H-4′), 3.61 (q, 1H, H-5′), 2.47 (q, 2H, CH_2-butyl), 1.57 (q, 2H, CH_2-butyl), 1.28 (q, 2H, CH_2-butyl), 0.85 (t, 3H, CH_3-butyl).

6.1.3 2′-O-Tosyl-8-bromoadenosine[63,65] (4).

Compound 3 (3.82g,6.7mmol) was dissolved in anhydrous MeOH (170ml). Triethylamine (14ml, 15 equiv.) and tosyl chloride (19.0g, 15 equiv.) were added and the resulting mixture was stirred at room temperature for 2 hours.The crystalline residue was filtered off and washed with anhydrous MeOH (3 X 50ml). (Yield 2.81g, 56mmol, 86%). A sample was recrystallised from MeOH; m.pt 195°C (Lit[63] 195°C). (Found: C, 41.10, H, 3.63; N, 14.30; $C_{17}H_{18}N_5O_6SBr$ requires C,40.81; H, 3.63; N, 14.00). I.R ν_{max} (KBr) 1152cm^{-1} (covalent tosylate); N.M.R : (d_6-DMSO) 7.95 (s, 1H, H-2), 7.54 (br, 2H, 6-NH_2), 7.37 (d, 1H, tosyl-Ha), 6.98 (d, 2H, Hb), 6.08 (d, 1H, OH-3′), 5.97 (d, 1H, H-1′), 5.72 (q, 1H, H-2′), 5.59 (t, 1H, OH-5′), 4.41 (br, 1H, H-3′), 4.10 (m, 1H, H-4′), 3.64 (m, 2H, H-5′), 2.27 (s, 3H,tosyl-CH_3).

6.1.4 2′-O-Tosyl-8-oxy-N^6,3′,5′-triacetyladenosine (5).

Compound 4 (2.0g, 4.0mmol) was dissolved in a mixture of AcOH (50ml) and Ac_2O (50ml) containing anhydrous NaOAc (6.0g). The solution was heated under reflux for 3 hours. The resulting mixture was subsequently decolourised with animal charcoal and filtered.The solvent was evaporated under reduced pressure and traces of Ac_2O were decomposed and removed by evaporation several times with EtOH (4 X 100ml). The residue was suspended in $CHCl_3$ and water (120ml of each). The organic layer was separated and washed with a saturated solution of $NaHCO_3$ (2 X 50ml), then with water (2 X 50ml) and then dried over anhydrous $MgSO_4$. Evaporation was performed under reduced pressure and a glassy compound was obtained, which on warming with EtOH yielded a white crystalline compound (3.2g, 70%); m.pt 204-205°C (Lit[63] 202-204°C). (Found: C, 48.63; H, 4.43; N, 12.38. $C_{23}H_{27}N_5O_{10}S$ requires C, 48.84; H, 4.81, N, 12.38); UV, λ_{max} (50% EtOH) 267nm.IR (KBr disc) : ν_{max} 1160cm^{-1} (covalent tosylate), 1720cm^{-1} (8-C=O); NMR:($CDCl_3$), 9.51 (br, 1H, N^7-H), 9.17 (br, 1H, N^6-H), 8.32 (s, 1H, H-2), 7.70 (d, 2H, tosyl-Ha), 7.19 (d, 2H, tosyl - Hb), 6.21 (d, 1H, H-1′), 6.09 (q, 1H,H-2′), 5.67 (m, 1H, H-3′), 4.34 (m, 2H, H-4′, 5′), 2.36 (s, 3H, NAc - Me), 2.30 (s, 3H, tosyl-Me), 2.05 (s, 6H, OAc-Me).

6.1.5 2′,8-O-Cycloadenosine[63,65,72] (6).

Compound 5 (3g, 5.3mmol) was dissolved in a mixture of anhydrous EtOH (50ml) and triethylamine (10ml, 15 equiv.). The mixture was heated under reflux for 60 hours, which resulted in the deacylation and cyclisation of compound 5. The solvent was

extracted under reduced pressure and 2′,8-O-cycloadenosine was deposited.(Yield 0.9g, 65%). For characterisation,a sample was recrystallised from ethanol; m.pt 208-210°C, compound becoming brown before melting (Lit[65] 208-209°C). UV, λ_{max} (EtOH), 266.5nm.(Found: C,43.97; H,4.96; N,25.95. $C_{10}H_{11}O_4N_5$ requires C,45.28, H, 4.18, N, 26.40).N.M.R (d_6-DMSO) 8.67 (s, 1H,H-2); 7.38 (s, 2H, N_6-H); 7.1 (d, 1H, H-1′); 6.21 (d, 1H, H-2′); 4.80 (br,1H, H-3′); 4.22 (br, 2H,H-4′,H-5′); 3.71 (v.br, 3H,-CH_2OH).

6.1.6 8-Hydrazino-arabinofuranosyladenosine[66,72,78] (7).

2′,8-O-Cycloadenosine (Compound 6) (1.0g, 3.37mmol) was heated under reflux with a 6-fold excess of hydrazine in absolute EtOH for 16 hours. When the solution was allowed to return to room temperature,white crystals of 8-Hydrazino-ara-A were deposited. The crystals were filtered off and the solvent was evaporated to dryness to leave a brown oily liquid, which was precipitated with water to give white crystals of the product (Total yield, 0.93g, 83%). M.pt 192-194°C (Lit[78] 192-194°C). UV,λ_{max}(MeOH) 264nm.(Found: C, 40.59; H, 5.12, N, 32.75. $C_{10}H_{15}N_7O_4$ requires C, 40.40; H, 5.05; N, 32.75). NMR (d_6-DMSO), 7.85 (s, 1H,H-2), 6.65 (s, 2H, N_6-H), 6.18 (d,1H, H-1′), 4.8 (br, 3H,H-2′,H-2′-OH, H-5′-OH), 4.22 (br, 3H, H-3′,4′,5′), 3.85 (s, 3H, -N_2H_3).

6.1.7 9-β-D-Arabinofuranosyladenosine[78] (Ara-A) (8).

8-Hydrazino-ara-A (Compound 7) (0.9g, 3.03mmol) was dissolved in an equivalent amount of anhydrous triethylamine and absolute ethanol (60ml). The resulting solution was heated under reflux for 60 hours under anhydrous conditions. The mixture

was allowed to return to room temperature and compound 8 was deposited. It was filtered off, washed with EtOH (25ml) and then dried (Yield 0.73g, 90%). For characterisation, a sample was recrystallised from EtOH; m.pt 264-266°C (Manufacturer's value 265-266°C). Found: C, 45.23; H, 4.90; N; 25.85; O, 24.02. $C_{10}H_{13}N_5O_4$ requires C, 44.94; H, 4.87; N, 26.22; O, 23.97). NMR (d_6-DMSO), 8.15 (s, 1H, H-2), 8.09 (s, 1H, H-8), 7.12 (s, 2H, N_6-H), 6.20 (d, 1H, H-1′), 5.56 (d, 1H, OH-2′), 5.48 (s, 1H, OH-3′), 5.02 (t, 1H, H-2′), 4.08 (t, 1H, H-3′), 3.66 (q, 2H, H_2-5′), 3.26 (s, 1H, OH-5′).

6.1.8 Preparation of 3′,5′-di-O-Tetraisopropyldisilyl-9-β-D-arabinofuranosyladenosine (3′,5′-di-O-TIPDS-Ara-A)[72,78] (9) from Ara-Adenosine (8).

Ara-A (Compound 8) (0.534g,2mmol) was dissolved in dry pyridine (20ml) and the solution was stirred in an inert and anhydrous atmosphere.1,1,3,3-tetraisopropyldisiloxane dichloride (0.632g, 0.64ml) was added dropwise with continuous vigorous stirring and the resulting mixture was stirred at room temperature for 3 hours. The pyridine was evaporated under reduced pressure and the residue was treated with a mixture of ethyl acetate (40 ml) and water (40 ml).The aqueous layer was separated and extracted with ethyl acetate (40ml). The organic layer was separated, washed with molar HCl (2 X 40ml), water (2x 40ml), saturated $NaHCO_3$ (40 ml), and finally with saturated NaCl (40ml) and then dried over $MgSO_4$. It was evaporated under reduced pressure to yield a foam (0.82g, 84%). For characterisation, a sample was purified by chromatography on a silica column (2x15cm), 20g

using MeOH:$CHCl_3$ (1:9) as eluent. The major fraction was collected. It was evaporated to yield compound 9. (M.pt 98-99°C) (Lit[72] 98-100°C). UV,λ_{max} 0.1N HCl, 257nm. (Found: C, 51.81; H, 7.37; N,13.80. $C_{22}H_{39}N_5O_5Si_2$ requires C, 51.83; H, 7.71, N, 13.73). NMR ($CDCl_3$), 8.18 (s, 1H, H-8), 8.10 (s, 1H, H-2), 6.13 (br, 2H,6-NH_2), 6.19 (d, 1H, H-1′), 5.28 (br, 1H, OH-2′), 4.65 (m, 2H, H-2′, H-3′), 4.02 (m, 2H, H-5′,5″), 3.85 (m, 1H, H-4′),1.2 (br,28H, iso-Prx4). MS *m/z* : 509 (M^+, (80%), 466 (M^+ -iso-Pr, 70%), 136 (Base+2, 10%), 135 (Base+1, 30%).

<u>6.1.9 2′-O-Triflyl-3′-5′-di-O-TIPDS-Ara-A [68,88] (10).</u>

3′,5′-di-O-TIPDS-Ara-A (Compound 9)(0.60g, 1mmol) was dissolved in anhydrous dichloromethane (10ml) and dimethylaminopyridine (DMAP) (0.12g, 1mmol) and anhydrous trimethylamine (1.6ml, 1.1 equiv.) were added. The solution was stirred in an inert atmosphere at room temperature until homogeneous (0.5hr). It was cooled to 5°C in an ice bath and trifluoromethanesulphonyl chloride (triflyl chloride) (0.11ml, 1mmol) was added dropwise keeping the temperature at 5°C. The mixture was allowed to gradually return to room temperature and left to stir overnight.TLC confirmed the completion of the reaction.The volume was reduced under vacuum and the oily residue applied to a column which was eluted by CH_2Cl_2:MeOH (87:13). The eluent was evaporated under reduced pressure to yield a foam. The foam solidified when dried over silica gel under reduced pressure (0.54g, 75%). M.pt 142-144°C. (Found: C, 43.06; H, 6.35; N, 9.99. $C_{23}H_{38}N_5O_7F_3SSi_2$ requires C, 43.04; H, 5.96; N, 10.91). UV,λ_{max} MeOH, 260nm. NMR ($CDCl_3$), 8.33 (s, 1H, H-8), 7.95 (s,

1H, H-2), 6.39 (d, 1H, H-1′), 5.73 (br, 2H, NH_2-6), 5.45 (m,2H, H-2′,3′), 4.2 (m, 2H, H-5′,5″), 4.02 (m, 1H, H-4′), 1.2 (br, 28H, iso-Prx4). MS *m/z* : 641 (M^+, 75%), 598 (M^+ - iso-Pr, 65%), 136 (Base+2, 5%), 135 (Base+1, 15%).

6.1.10 2′-Azido-2′-deoxy-3′,5′-di-O-TIPDS-Ara-A[76] (11).

Compound 10 (0.43g,0.67mmol) was dissolved in anhydrous dimethylformamide (8ml). Sodium azide (0.045g, 0.67mmol) was added and the resulting solution was stirred at room temperature for 3 hours. The mixture was then poured into ice water (40ml) and the white suspension extracted with ethyl acetate (100ml). The organic layer was washed with water (75ml), dilute HCl (75ml) and saturated $NaHCO_3$ (75ml). The solvent was removed under reduced pressure to yield an oil which was crystallised from petroleum ether (80-100°).A white amorphous solid was obtained (0.30g, 82%); m.pt 168-170°C (Lit[76] 168-170°C). (Found: C, 49.53; H, 7.08; N, 20.98. $C_{22}H_{38}N_8O_4Si_2$ requires C, 49.41; H, 7.16; N, 20.96). UV, λ_{max} $CHCl_3$,260nm. IR, ν_{max} KBr, 2110cm^{-1}, N_3. NMR ($CDCl_3$), 8.32 (s, 1H, H-8), 8.02 (s,1H, H-2), 5.76 (s, 1H, H-1′), 5.55 (br, 2H, NH_2-6), 5.19 (dd,1H, H-3′), 4.61 (d, 1H, H-2′), 4.1 (m, 3H, H-4′, 5′, 5″), 1.2 (br, 28H, iso-Prx4).

6.1.11 2′-Azido-2′-deoxyadenosine [67] (12).

Deprotection of compound 11 was effected by the addition of 2 equivalents of tetrabutylammoniun fluoride (TBAF) in THF to yield 2′-azido,2′-deoxyadenosine.

Compound 11 (0.27g,0.5mmol) was dissolved in tetrahydrofuran (10ml) and TBAF (2 equivalents) was added and the reaction

mixture was maintained at 75°C for 1 hour. The THF was evaporated under reduced pressure and the residue was dissolved in 10% methanol/water which was then applied to a column (1.2X 16cm) of Dowex 1-X 2 (OH^-) resin. Elution was effected by 50% methanol-water (R_F 0.55). Evaporation of the solvent yielded the crystalline compound 12 (0.11g, 75%); m.pt 218-221°C (Lit[76] gives a variety of values). (Found: C, 40.60; H, 4.11; N, 36.40. $C_{10}H_{12}N_8O_3$ requires C, 41.09; H, 4.13; N, 38.34). UV, λ_{max} water 259.5nm. IR, ν_{max} KBr, 2110cm^{-1}. NMR (DMSO-d_6), 8.41 (s, 1H, H-8), 8.16 (s, 1H, H-2), 7.38 (br, 2H, NH_2-6), 6.10 (d, 1H, H-1′), 6.05 (d, 1H, OH-3′), 5.25 (t, 1H, OH-5′), 4.65 (dd, 1H, H-3′), 4.02 (m, 1H, H-4′), 3.65 (m, 2H, H-5′,5″).

6.1.12 Attempted Preparation of 2′-Azido-2′-deoxy-3′,5′-di-O-IIPDS-Inosine from 2′-Azido-2′-deoxy-3′,5′-di-O-IIPDS-Ara-A(11).

Compound 11 (0.54g, 1.0mmol) was dissolved in absolute ethanol (15ml) and stirred continuously at room temperature while isopropyl nitrite (3eq, 0.27g) was added. Glacial acetic acid (3eq, 0.18ml) was then added dropwise and the reaction was followed by TLC. Once all the acid was added the reaction was stirred at room temperature and monitored by TLC for the formation of product at 3 hourly intervals initially and then left to stir overnight. TLC showed only one compound - the starting material after 24 hours. The mixture was heated gently under reflux and the reaction was followed by TLC on an hourly basis. No product was formed. The starting material (Compound 11) was recovered, dissolved in absolute ethanol and

stirred with sodium nitrite (3eq, 0.21g) in a minimum amount of water. HCl (0.5M, 2ml) was added dropwise and the mixture was stirred vigorously for another 2 hours once addition was completed. TLC showed the presence of starting material only and showed no product. Gentle heating under reflux for another 2 hours did not give any product.

SCHEME II

6.2.1 3'-5'-di-O-TIPDS-Adenosine[72-74] (13).

Adenosine (13.35g, 50mmol) was dissolved in dry pyridine (250ml) in an inert atmosphere. 1,1,3,3-Tetraisopropyldisiloxane dichloride (TIPDS-Cl_2) (15.8g, 16ml) was added dropwise at room temperature with vigorous stirring. Once addition was completed, the reaction mixture was allowed to stir for 3 hours until all solid particles had reacted. The pyridine was removed under reduced pressure and the residue was dissolved in a mixture of equal volumes of ethyl acetate/water (300:300ml). The organic layer was separated and washed with molar HCl (2 X 300ml), water(300ml), saturated $NaHCO_3$ (300ml) and saturated NaCl (300ml) and finally dried over $MgSO_4$. Ethyl acetate was removed under reduced pressure.The syrupy residue was dissolved in hot acetonitrile. Evaporation of the acetonitrile under reduced pressure yielded a white crystalline solid. (Yield 24.65g, 90%).For characterisation,a sample was recrystallised from hot acetonitrile; m.pt 98-99°C (Lit[73] 98.5-99.5°C). (Found: C, 50.91; H, 7.71; N, 13.23. $C_{22}H_{39}N_5O_5Si_2$ requires C, 51.83; H, 7.71, N, 13.73). UV, λ_{max} 0.1M HCl, 257nm. NMR

(DMSO-d_6), 8.19 (s, 1H, H-8), 8.10 (s, 1H, H-2), 7.30 (br, 2H, NH_2-6), 5.86 (d, 1H, H-1′), 5.60 (d, 1H, OH-2′), 4.78 (s,1H, H-3′), 4.05 (m, 2H, H-5′,5″), 3.9 (m, 1H, H-4′), 1.2 (br, iso-Pr X 4)

6.2.2 Preparation of 3′,5′-di-O-TIPDS-Ara-A^{81}(9) from 3′,5′-di-O-TIPDS-Adenosine (13).

An oxidising complex consisting of CrO_3/Py/Ac_2O was prepared by dissolving CrO_3 (3g) in anhydrous pyridine (5ml) and cooling the resulting yellow sludge to below 0°C. Ac_2O was added dropwise with vigorous stirring while maintaining the temperature below freezing. The resulting brown compound was dissolved in previously dried dichloromethane (60ml) and the solution stirred until all particles were dissolved. 3′,5′-di-O-TIPDS-adenosine (Compound 13) (5.09g, 10mmol) was added to the complex in small amounts while maintaining vigorous stirring. Once the addition was completed, the mixture was stirred for 45mins at room temperature. Ethyl acetate (200ml) was added and the mixture was filtered through neutral silica gel to remove all the fine particles. The filtrate was evaporated under reduced pressure while maintaining the temperature below 25°C. The residue was dissolved in 95% ethanol (100ml) and sodium borohydride (1.60g dissolved in 15ml of water) was added dropwise with vigorous stirring while maintaining the temperature of the solution under 25°C. Once all the borohydride solution was added, the mixture was stirred for another 45min. The resulting bright yellow solution was poured into a saturated solution of NaCl and then extracted with ethyl

acetate. The organic layer was washed with additional saturated NaCl and dried over $MgSO_4$. On evaporation under reduced pressure, an oil was obtained. Thin layer chromatography showed two spots - TIPDS-adenosine and TIPDS-Ara-A (R_F 0.57 and 0.32 respectively). The oil was applied to a column of silica gel (2x 20cm, 30g) and eluted with a mixture of chloroform:methanol(96:4). On evaporation of the solvent, a pale yellow foam was obtained. It showed all the chemical characteristics of 3′,5′-di-O-TIPDS-Ara-A (See Section 6.1.8).

SCHEME III

6.3.1 2′,3′,5′-Triaceto-Inosine (16).

Inosine (2g, 7.50mmol), was dissolved in a mixture of anhydrous acetic anhydride (12ml), glacial acetic acid (12ml) and anhydrous sodium acetate (2g). The resulting mixture was heated under reflux under anhydrous conditions for 2 hours.The solvent was evaporated under reduced pressure to yield a clear gum. The gum was dissolved in hot ethanol, which was removed under reduced pressure to give a white highly crystalline material. The product was dissolved in hot water and then allowed to cool down. Inosine triacetate crystallised out and was filtered off (Yield 2.65g, 91.4%); m.pt 233-234°C.(Found: C, 48.61; H, 4.66; N, 14.01.$C_{16}H_{18}N_4O_8$ requires C, 48.73, H, 4.6,N, 14.20). UV,λ_{max} EtOH 256.5nm. NMR ($CDCl_3$), 8.20 (s,1H, H-8),7.85 (s, 1H, H-2), 6.35 (d, 1H, H-2′), 5.85 (t, 1H, H-1′), 5.70 (m, 1H, H-3′), 4.24 (s, 2H, H-4′,5′), 2.46, 2.25 (2s, 9H,Ac,6; Ac,3).

6.3.2 6-Chloro-2′,3′,5′-triaceto-Inosine[80,81] (17).

Inosine triacetate (Compound 16) (2.25g, 6.20mmol) was dissolved in a mixture of acetonitrile (40ml) and dimethylaminopyridine (DMAP) (2.5eq, 2.13g).The mixture was stirred for 0.5h at room temperature. Phosphorus oxychloride (5eq, 4ml) was added and the mixture was stirred for another 2 hours. The mixture was then heated under reflux for 2 hours. The solvent was removed under reduced pressure and the residue was dissolved in cold water (50ml). The resulting aqueous layer was extracted with ethyl acetate (75ml). The colourless organic extract was washed successively with molar HCl (50ml), saturated sodium bicarbonate (50ml),water (50ml) and saturated sodium chloride and then dried over magnesium sulphate. The ethyl acetate was then removed under reduced pressure. A clear gum was obtained as product (Yield 2.15g, 90%). Attempts to crystallise the gum gave side products as shown by analysis of the crystallised product.The gum gave one compound on TLC, R_F value 0.44 (CH_2Cl_2:MeOH,90:10),while the recrystallised product gave more than two compounds which were different to the gum product.All analyses were carried out on the gum.(Found: C, 46.80; H,4.29; N,12.11. $C_{16}H_{17}N_4O_7Cl$ requires C, 46.55;H,4.15, N, 13.57).UV,λ_{max} 50%-EtOH 268nm.NMR ($CDCl_3$), 8.80 (s,1H,H-8), 8.00 (s,1H, H-2), 6.57 (d, 1H, H-1′), 6.07 (t, 1H, H-3′), 5.92, (m,1H, H-2′), 4.24(s, 2H, H-4′,5′), 2.46,2.25 (2s,9H,Ac,6;Ac,3).

6.3.3 N^6,N^6-Dimethyladenosine (18).

6-Chloro-inosine-triacetate (compound 17) (2.0g,7.5mmol) was treated with an excess of 40% aqueous dimethylamine at room

temperature and stirred for two hours, when a white amorphous solid curdled. The solid was filtered off and recrystallised from hot methanol. (Yield 0.58g, 36%). A second crop of dimethyladenosine was obtained by removing the solvent under reduced pressure and dissolving the residue in dry methanol and stirring with sodium methoxide (0.5g) until further compound curdled out.(Yield 0.14g, 8.6%); m.pt 184-186°C. (Identical to commercial sample. A sample consisting of the commercially available compound and the above product gave the same melting point).(Found: C, 48.71; H, 5.90; N, 23.76. $C_{12}H_{17}N_5O_4$ requires C,48.80; H,5.80; N, 23.76). NMR ($CDCl_3$), 8.32 (s, 1H, H-8), 8.20 (s,1H, H-2), 5.90 (d, 1H, H-1′), 5.40 (m, 1H, OH-3′), 5.18 (d, 1H,OH-2′), 4.60 (m, 1H, H-2′), 4.18 (m, 1H, H-3′),3.95 (s,1H, H-4′), 3.62 (br, 2H, H-5′,5″),3.42 (br, 1H, OH-5′), 3.23 (s,6H, N^6,N^6-DiMe). MS *m/z* : 296 (M^+, 40%), 164 (B+1, 18%), 133 (sugar+1, 5%).

6.3.4 Reaction between N^6,N^6-Dimethyladenosine(18) and 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane(TIPDS-Cl_2)(28): Formation of 2′,3′-di-O-TIPDS-N^6,N^6-Dimethyladenosine(19).

Dimethyladenosine (Compound 18) (0.5g,0.53ml,1.5mmol) was dissolved in dry pyridine (10ml) and the solution was stirred in an inert atmosphere until all particles were dissolved. TIPDS-Cl_2 (0.5g,1.5mmol) (Compound 28) was added and the mixture was stirred overnight. The solvent was removed by distillation and the residue was dissolved in water (15ml).The aqueous layer was then extracted with ethyl acetate (25ml).The organic layer was separated and washed successively with molar

HCl (15ml), water (15ml), a saturated solution of sodium bicarbonate (15ml) and a saturated solution of sodium chloride(15ml) and then dried over anhydrous magnesium sulphate. The solvent was then removed under reduced pressure to give 2′,3′-di-O-TIPDS-N^6,N^6-dimethyladenosine (Compound 19) as a white foam. The foam solidified when dried over silica gel under reduced pressure (Yield 0.73g, 92%). M.pt 94-96°C. (Found: C, 52.50; H, 8.06; N, 14.53; $C_{24}H_{43}N_5O_5Si_2$ requires C, 53.59; H, 8.05, N, 13.02). NMR ($CDCl_3$) 8.48 (s,1H, H-8), 8.36 (s, 1H, H-2), 6.25 (d, 1H, H-1′), 4.32 (br m, 5H, H-1′,H-2′,H-3′, H-4′, OH-5′), 4.00 (br, 2H, H-5′ ,5″), 3.45 (s, 6H, N^6,N^6-Me_2), 1.12 (br, 28H, iso-Pr X 4). MS *m/z* : 538 (M^+, 70%), 495 (M^+ - iso-Pr, 26%), 164 (Base+1, 36%), 165 (Base+2, 12%).

6.3.5 3′,5′-di-O-TIPDS-Inosine.

Inosine (5.36g, 20mmol) was dissolved in dry pyridine (150ml) and the mixture was stirred in an inert atmosphere until all solid particles had dissolved. 1,3-Dichloro-1,1,3,3-dichlorotetraisopropyl disiloxane (6.32g, 6.4ml) was added dropwise and the mixture was stirred at room temperature for 24 hours. The pyridine was evaporated under reduced pressure and the residue was dissolved in a mixture of equal volumes of water and ethyl acetate. The organic layer was separated, washed successively with molar HCl (100ml), water (100ml), saturated sodium bicarbonate (100ml) and saturated sodium chloride (100ml) and dried over magnesium sulphate. The ethyl acetate was removed under reduced pressure to leave the product (8.75g, 85%); m.pt 105-107°C. (Found: C, 51.80; H, 7.37; N, 9.75. $C_{22}H_{38}N_4O_6Si_2$

requires C, 51.76; H, 7.45; N, 10.50. NMR (DMSO-d_6), 8.18 (s, 1H, H-8), 8.02 (s, 1H, H-2), 5.88 (d, 1H, H-1′), 5.70 (m, 1H, OH-2′), 4.60 (dd, 1H, H-3′), 4.45 (dd, 1H, H-2′),4.10 (s,1H,H-5′), 4.04 (m, 1H, H-4′), 3.95 (s, 1H, H-5″), 1.20 (br, 28H, iso-Pr).

6.3.6 2′-Aceto-3′,5′-di-O-TIPDS-Inosine.

3′,5′-di-O-TIPDS-Inosine (1.86g, 3.65mmol) was suspended in a mixture of acetic acid (10ml), acetic anhydride (10ml) and sodium acetate (2.5g). The mixture was heated under reflux for 1 hour. The solvent was evaporated under reduced pressure and the acetic anhydride was decomposed with small amounts of ethanol. The white crystalline solid residue was dissolved in water and extracted with ethyl acetate. The organic layer was separated and washed successively with molar HCl (25ml), water (25ml), a saturated solution of sodium bicarbonate (25ml) and a saturated solution of sodium chloride and then dried over anhydrous magnesium sulphate.The solvent was removed under reduced pressure to give a white crystalline solid (Yield 1.68g, 83%). M.pt 173-175°C. (Found: C, 51.99; H, 7.27; N, 10.24. $C_{24}H_{40}N_4O_7Si_2$ requires C, 52.17; H, 7.25; N, 10.14). UV,λ_{max} EtOH 272nm. NMR ($CDCl_3$) 8.20 (s, 1H, H-8), 8.10 (s, 1H, H-2), 6.08 (s, 1H, H-1′), 5.68 (d, 1H, H-2′), 4.86 (m, 1H, H-3′), 4.12 (br, 3H, H-4′, 5′,5″), 2.16 (s, 3H, Ac), 1.12 (br, 28H, iso-Pr).

6.3.7. Reaction of 2′-Aceto-3′,5′-di-O-TIPDS-Inosine with Phosphorus Oxychloride[80,81].

The compound from the previous reaction (1.3g,2.4mmol) was dissolved in a mixture of anhydrous acetonitrile (20ml) and dime-

thylaminopyridine (DMAP) (2eq.,0.60g). The mixture was stirred for 0.5 hour at room temperature.Phosphorus oxychloride (4eq., 0.9ml) was added and the mixture was stirred for another 3 hours. The mixture was then heated under reflux for a further 3 hours and the reaction was monitored by TLC. No product was detected. The reaction mixture was then heated under reflux overnight but no reaction took place. This reaction is analogous to that used for the preparation of 6-chloro-2',3',5'-triacetoinosine (See 6.3.2) in which case the reaction did proceed. Lee et al[55] encountered similar problems when they used different protective groups.

SCHEME IV

6.4.1. 3,4-O-Benzylidene-D-ribono-1,5-lactone[60,61](21).

D-Ribono-1,4-lactone (20g, 0.135mole) was dissolved in benzaldehyde (200ml) and concentrated hydrochloric acid (20ml) was added. The resulting suspension was vigorously shaken at room temperature for 3 hours : the starting material dissolved in several minutes and then the product slowly crystallised from the solution.The mixture was diluted with ether (250ml) and the product filtered off. The crude product was washed with ether (500ml), a saturated aqueous solution of sodium bicarbonate (200ml), water (200ml) and finally more ether (500ml) and then dried over phosphorus pentoxide to yield the product as a crystalline solid (28.33g, 89%).For characterisation a small sample was recrystallised from hot ethyl acetate;m.pt 233-235°C (Lit[61] 233-235°C).(Found:C, 60.76; H,5.09. $C_{12}H_{12}O_5$ requires C,61.02;

H,5.08). NMR (DMSO-d_6) 7.42 (m, 5H, ArH), 5.80 (s, 1H, PhCH), 4.75 (m, 3H, H-2,3,4), 4.33 (d, 1H, H-5), 4.20 (d, 1H, H-5).

6.4.2 3,4-O-(R)-Benzylidene-2-O-trifluoromethanesulphonyl-D-ribono-1,5-lactone (22).

Compound 21 (13.96g, 59.1mmol) was suspended in dry pyridine (150ml) and the reaction mixture was cooled to -10°C in an inert atmosphere. Trifluoromethanesulphonic anhydride (1.2 equiv., 20g) was added dropwise to the vigorously stirred mixture while maintaining the temperature below -10°C. Once addition was completed, the resulting solution was allowed to return to room temperature and was stirred at this temperature for one hour. The pyridine solution was diluted with ethyl acetate (300ml),then washed successively with water (300ml) and a saturated solution of sodium bicarbonate (300ml) and dried over magnesium sulphate. The solvent was removed under reduced pressure to give the required triflate derivative as a yellow solid which was used without further purification in the next stage {(synthesis of the azido-lactone (compound 23)}. For characterisation a small amount of the triflate was recrystallised from ethanol to yield white needles; m.pt 172-173.5°C (Lit[84] 172-173°C).IR, ν_{max} (nujol) 1770, 1455, 1410 and 1090 cm^{-1}.(Found: C, 42.41; H, 2.97. $C_{13}H_{11}F_3O_7S$ requires C, 42.39; H,2.97).NMR (DMSO-d_6) 7.46 (m, 5H, ArH), 5.87 (s,1H,PhCH), 5.35 (d,1H, H-2, J_{23} 3.4Hz), 5.00 (dd,1H, H-3,J_{34} 8.0 Hz), 4.79 (d,1H, H-4), 4.68 (d,1H, H-5,J_{55} 13.6Hz), 4.38 (dd,1H,H-5, J_{45} 1.7Hz).

6.4.3 2-Azido-3,4-O-(R)-benzylidene-2-deoxy-D-ribono-1,5-lactone (23).

Compound 22 in its crude form was dissolved in DMF (100ml) and stirred with sodium azide (6.4g, 98.5mmol) at room temperature for 1.5 hour.The mixture was poured into ice-water (200ml) and the precipitate was filtered off. It was dissolved in ethyl acetate (300ml), washed with water (300ml) and dried over magnesium sulphate. The organic solvent was removed under reduced pressure and the residue was dissolved in hot ethanol. The title compound (compound 23) crystallised out on cooling (Yield 9.8g, 64%); m.pt 145°C with decomposition (Lit[84] 145°C). (Found: C, 55.08; H, 4.21; N, 16.09. $C_{12}H_{11}N_3O_4$ requires C, 55.17, H, 4.21, N, 16.09). IR_{KBr}, ν_{max} 2110, 1755, 1460,1405, 1160 and 1090 cm^{-1}. NMR (DMSO-d_6) 7.45 (m,5H, ArH), 5.78 (s, 1H, PhCH), 4.91 (dd, 1H, H-3, J_{34} 8.0 Hz), 4.67 (dd, 1H, H-4), 4.61 (d, 1H, H-5, J_{55} 13.4 Hz),4.25 (dd, 1H, H-5, J_{45} 1.7 Hz), 3.95 (d, 1H, H-2).

6.4.4 2-Azido-2-deoxy-D-ribono-1,4-lactone (24).

The protected azido-lactone (compound 23) (5g, 19.2mmol) was dissolved in a mixture of trifluoroacetic acid (26ml) and water (13ml) by stirring the solution at 50°C for 1.5 hour. The solvent was evaporated under reduced pressure and the residue was dissolved in a minimum amount of a mixture of ethyl acetate and hexane (3:2) and separated by flash chromatography.The product (Compound 24) was obtained as a white solid (Yield 3.1g, 94%). It was recrystallised from ether; m.pt 84-85°C (Lit[84] 85°C). (Found: C, 34.74; H, 4.03; N, 24.15. $C_5H_7N_3O_4$ requires C,

34.68; H,4.05; N,24.28). IR_{KBr}, ν_{max}, 3400 (br),2105,1765cm^{-1}. NMR (Me_2CO-d_6) 5.28 (br d, 1H, D_2O exchange, OH), 4.61 (d, 1H, H-3, J_{23} 5.3 Hz), 4.50 (t, 1H, H-4), 4.44 (d,1H, H-2), 3.83 (d, 2H, H-5, 5', J_{45} 3.0 Hz), 2.98 (br, s, 1H, D_2O exchange OH).

6.4.5 Reaction between 2-Azido-2-deoxy-D-ribono-1,4-lactone(24) and TIPDS-Cl_2.

Compound 24 (0.17g, 1mmol) was dissolved in pyridine (5ml) and the solution was stirred continuously in an atmosphere of nitrogen. TIPDS-Cl_2 (0.31g, 0.35ml) was added dropwise and the mixture was stirred for a further 3 hours at room temperature. The reaction workup was carried out in a similar manner to that described in experiments 6.1.8 (Scheme I), 6.2.1 (Scheme II) and 6.3.5 (Scheme III). An oil was obtained and it was subjected to NMR and IR spectroscopic analysis. The analytical results showed complete loss of the structure of the starting material but they indicated strongly the presence of the TIPDS group. It was presumed that the ring structure had opened. Fleet[84] and Scriven[85] reported similar problems while attempting to protect the hydroxyl functions of the lactone with different protective groups.

6.4.6 Catalytic Reduction of 2-Azido-2-deoxy-D-ribono-1,4-lactone (24).

The azido-ribonolactone (Compound 24)(1.07g, 6.17mmol) was dissolved in ethyl acetate (30ml) and the solution was stirred at room temperature in a hydrogen atmosphere in the presence of palladium black (0.3g). After 24 hours, the ethyl acetate was

removed by distillation and the residue was suspended in water (90ml). Aqueous sodium hydroxide (2M, 5.2ml, 8.4mmol) was added and the suspension was stirred for 24 hours until only the catalyst remained undissolved. The solution was then filtered and evaporated and the brown syrupy residue was purified by chromatography (eluted with aqueous pyridine) to give a whitish solid, which after recrystallisation from hot ethanol yielded a white solid (0.63g).The compound melted with decomposition at 247°C. IR_{KBr}, ν_{max} gave 3401, 3099, 3036, 2927, 2713, 2565, 2427, 1652, 1615, 1568 cm^{-1}. ^1H NMR (D_2O) d, 4.25-4.2 (2H, m, H-3, 4); 3.85 (1H, d, H-2, J_{23} 5.0 Hz); 3.41 (1H, dd, H-5', $J_{45'}$ 4.9 Hz); 3.17 (1H, dd, H-5, J_{45} 4.2 Hz, $J_{55'}$ 12.4 Hz).^{13}C NMR (D_2O) d 48.21 (t, C-5); 64.13 (d, C-2); 69.77 (d, C4); 73.92 (d,C-3); 171.9 (s, C-1). *m/z* (FAB+) :148 ($M + H^+$, 100%). (Found: C, 40.90; H, 6.28; N, 9.84). This compound was sent to Dr G.W.J Fleet, Dyson Perrins Laboratory, Oxford University for interpretation of NMR spectra and it was identified as (2R),(3S),(4R)-3,4-dihydroxyproline.

SCHEME V

6.5.1 Grignard Reagent - Isopropylmagnesium Chloride[86] (25).

All apparatus was rigorously cleaned and left in a hot oven overnight. Diethyl ether was left over calcium hydride for 48 hours and then distilled using a fractionating column and stored over sodium wire.Isopropyl alcohol was dried over phosphorus pentoxide and distilled using a fractionating column. Magnesium turnings were left in an oven at 100°C overnight. Magnesium turnings (60.80g, 2.5 moles) were placed in a 2l

3-neck round bottom flask and diethyl ether (600ml) was added. The flask was fitted with a stirrer, a water condenser fitted with a drying tube containing silica and a dropping funnel containing isopropyl chloride (196.25g, 228.5ml, 2.5 moles). To initiate the reaction, isopropyl chloride (25ml) was allowed to flow into the flask and iodine crystals were added. Gentle warming was supplied by a hair dryer. Once the reaction had started, the isopropyl chloride was added dropwise at such a rate that the ether boiled gently. Once the addition was completed, the dropping funnel was removed and the mixture was well protected against moisture, and stirred for 6 hours to allow all magnesium particles to dissolve.

6.5.2. Di-isopropylsilanol (26).

Isopropylmagnesium chloride (Compound 25) (2.25 moles in 1.8l of ether) was stirred and cooled to -20°C using a methanol bath cooled with liquid nitrogen.Trichlorosilane (0.50 moles,68.0g, 51.0ml) in ether (150ml) was added dropwise while maintaining a temperature of -15 to -20°C. Once addition was completed, the mixture was stirred for 15 hours at room temperature and heated under reflux for a further 24 hours. The resulting emulsion was then poured into a mixture of ice and molar hydrochloric acid, while keeping the temperature below -5°C during the acid hydrolysis. The ethereal fraction was separated and dried over magnesium sulphate and the ether was removed by distillation to leave an oil. The oil was fractionally distilled under reduced pressure in an inert atmosphere to yield the title compound (Yield 66g,84%).B.pt 74-75°C (6mm Hg) (Lit[86] 73-74°C at 6mmHg).

6.5.3 1,1,3,3-Tetraisopropyldisiloxane (TIPDS)(27).

Phosphorus pentoxide (71g, 0.5mole) was added to di-isopropylsilanol (compound 26) (66g, 0.5 mole) and the mixture was allowed to stand for one hour. The mixture was then fractionally distilled under reduced pressure in an inert atmosphere. The distillate, 1,1,3,3-tetraisopropyldisiloxane was collected (27g, 84%). B.pt 104-105°C (10mm Hg) (Lit[73] 104°C at 10mmHg). IR thin film ν_{max} 2150cm^{-1} (Si- H bond).

6.5.4 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[70] (TIPDS-Cl_2) (28).

Compound 27 (25g,0.1 mole) was dissolved in anhydrous carbon tetrachloride (0.5l) in a 3-neck round bottom flask fitted with a delivery tube through which chlorine gas was continuously bubbled. One neck was fitted to a water pump while the third neck carried a calcium chloride drying tube. The flask was cooled in an ice bath and the solution was stirred vigorously. The water pump was switched on and dry chlorine gas was bubbled through for 15-20 minutes, during which time the solution underwent an exothermic reaction and the solution became persistently yellow. The chlorine flow was stopped and any dissolved HCl gas was allowed to evacuate.The organic solvent was removed under reduced pressure in an inert atmosphere to yield an oily residue which was stored under nitrogen. The oil was distilled under reduced pressure to afford the title compound which was stored under nitrogen. (Yield 22.4g,71%). B.pt 118-120°C (15mm Hg). IR thin film - absence of band at 2150cm^{-1} (Si-H bond). The IR spectrum was identical to that

for the commercial product.

CHAPTER 7

CONCLUSION

'' *Two principles are necessary so that life shall succeed : one consists of proteins, and the other of nucleic acids*''.

These were the words used by Professor Thorell in 1959 in introducing the two Nobel prize winners in physiology and medicine. They characterise very appropriately the key position of the two cell constituents which must be present for any living cell to function. The proteins (mostly the functional, i.e hormones) have been exploited to their limits by scientists. Similarly there is a new horizon for the nucleoside chemists to exploit these molecules which are showing increasing clinical significance as powerful antiviral and anti-cancer agents. One common nucleoside - a household name which has revolutionised the treatment of the human immunodeficiency virus (HIV) with very good success, is 3'-azido,3'-deoxythymidine (AZT). The high cost of these compounds is reflected in the difficulties of their synthesis.

We were determined to produce our target compound irrespective of the route chosen; this is shown by the different approaches made. It was our belief that the compound has enormous potential - a belief shared by the scientists at Glaxo who were prepared to test its biological viability. As others had experienced in the past, the functionalisation of two different chemical entities - a base and a sugar, poses many synthetic problems. We believed strongly that the use of TIPDS as a

protecting group would solve many problems experienced by other chemists in their selection and usage of protecting groups.

With the benefit of hindsight, using non-scientific arguments, it can be deduced from the experiments carried out in this study that the TIPDS did produce some stereochemical effect on the nucleoside so as to render the base part of the molecule chemically inactive. Further research is required on this topic of TIPDS-nucleoside comformations. The only work available on the conformation of TIPDS compounds is that of Robins[81], who looked at the anomeric configuration with no mention of its effect on chemical reactivity.The few chemists who have used TIPDS as a protecting group were involved only in the functionalisation of the sugar moiety, leaving the base molecule untouched. Our study was novel in that it did not only involve the syntheses of novel compounds, but it also included a novel method of base functionalisation, thus extrapolating further on the use of TIPDS as a protecting group.

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