THE SYNTHESIS OF NUGLEOSIDE AND SILYL NUCLEOTIDE ANALOGUES

A Thesis

by

© James Cormier

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ABSTRACT

A route to the synthesis of arabino and xylonucleosides is described. This route takes advantage of highly selective ribonucleoside hydroxyl protection procedures which have recently been developed. The route is straightforward and broadly applicable. It may be applied to both purine and pyrimidine nucleosides. Synthesis, deprotection, and characterisation of the target compounds are described. The work is compared to that of others in the field.

A novel class of oligonucleotide analogues is described. In this group, the phosphorus atom of the internucleotide link is replaced by silicon. The synthesis of both oligothymidine and oligo-2'-deoxyadenosine nucleotide analogues of this class is described. Various substituents at silicon are employed, and oligonucleotide analogs of up to six units long are synthesised, characterised and deprotected. The circular dichroism spectra of the deprotected hexamers is presented.

LA SYNTHESE D'ANALOGUES DE NUCLEOSIDES ET DE NUCLEOTIDES SILYLES

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RESUME

Une voie de synthèse d'arabino et de xylonucléosides est décrite. Cette méthode exploite certaines procédures hautement sélectives récemment développées pour la protection des groupements hydroxyles de ribonucléosides. La méthode est simple et directe tout en étant propre à de multiples applications. Elle est utile à la fois pour la préparation de nucléosides puriques et pyrimidiques. La synthèse, la déprotection et la caractérisation des composés sont décrites et comparées à celles rapportées dans la littérature.

Une nouvelle classe d'analogues de nucléotides est décrite. Dans ces composés, l'atome de phosphore impliqué dans le lien internucléotidique est remplacé par un atome de silicium. La synthèse de tels analogues d'oligothymidine et d'oligodésoxy-2' adénosine est décrite. Divers substituants de l'atome de silicium sont employés lors de la préparation de ces composés. La synthèse, la caractérisation et la déprotection d'analogues possédant jusqu'à six nucléotides sont décrites.. Les spectres de dichroisme circulaire des hexamèrs déprotégés sont aussi présentés.

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for Rose

Acknowledgements

I would like to express my gratitude and appreciation to Dr. Kelvin Ogilvie, for his supervision, advice, financial support and patience during this work.

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Abbreviations

A	adenosine
Ad	adenine
Ad^{Bz}	N^6-benzoyladenine
Ac_2O	acetic anhydride
ara	arabinofuranosyl
B	heterocyclic nucleoside base
b. p.	boiling point
bs	broad singlet
bm	broad multiplet
Bz	benzoyl
C	cytidine
C^{Bz}	N^4-benzoyl cytosine
CD	circular dichroism
cm	centimeter
d	doublet
D	deuterium
dA	2'-deoxyadenosine
DABCO	1,4-diazobicyclo[2.2.2]undecane
DCC	dicyclohexylcarbodiimide
DCU	dicyclohexylurea
dd	doublet of doublets
^oC	degree Celsius
DMAP	4-(N,N-dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DMT	4,4'dimethoxytrityl
DNA	2'-deoxyribonucleic acid
Et	ethyl
FAB	fast atom bombardment
G	guanosine
g	gram
h	hour

i	*iso*
J	coupling constant
l	litre
Lv	levulinyl
lxyo	lyxofuranosyl
m	multiplet
M	molar (mole l^{-1})
u	micro
m	mass
Me	methyl
mg	milligram
min	minute
ml	millilitre
MS	mass spectrum
ul	microlitre
mmol	millimole
MMT	monomethoxytrityl
m. p.	melting point
mRNA	messenger RNA
nm	nanometer
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
Pr	propyl
py	pyridine
Rf	relative chromatographic mobility
RNA	ribonucleic acid
rRNA	ribosomal RNA
s	singlet
sec	second
t	triplet
t	*tert*
TCA	trichloroacetic acid
TLC	thin layer chromatography

tRNA	transfer RNA
U	uridine
UV	ultraviolet
v	volume
w	weight
xylo	xylofuranosyl
z	ionic charge

General introduction.

Man has always sought to understand and control the world around him, in order to bend it to his purpose. One of his earliest achievements was to learn to breed plants and animals to bring out desirable traits and suppress unwanted characteristics. In this way, he was able to produce plants and animals for food which were fast-growing and high-yielding. Animals were also bred as work mates. This freed man from a hunter-gatherer existence, a life in the natural state which Thomas Hobbes was to describe as "nasty, brutish and short". Once freed from the necessity of spending every waking moment in the pursuit of food and shelter, man was able to settle down and develop societies and cultures that we today call civilised.

Although early man was able to manipulate the characteristics of his plants and animals in desired ways, he did not understand at all the mechanism of the process. This state of affairs persisted until nearly the present day.

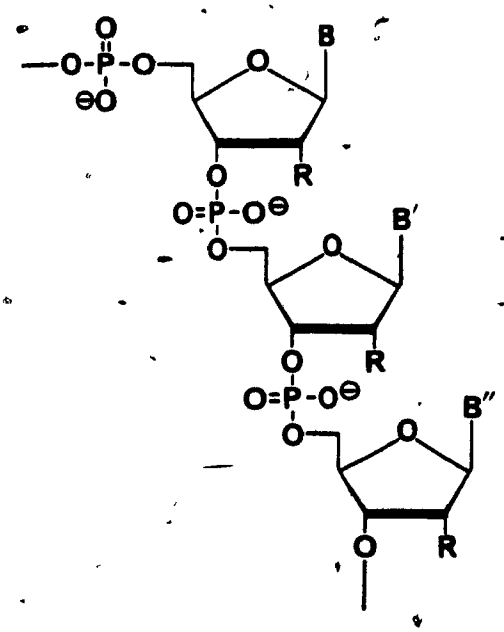
The revolutionary changes in Western society wrought by the Renaissance brought forth an interest in scientific research into the nature of the world around us. This drive has sustained us to the present day. One of the areas heavily studied in the past two centuries has been the mechanism of heredity. The monk Gregor Mendel, working with plants, was able to demonstrate that some traits are passed on from one generation to the next in apparently discrete units and in a fashion predictable by simple statistical laws. Although he did not know the nature of these units of heredity, the principle had been enunciated.

Meanwhile, by the early 1870s, the German researcher Miescher[1,2], had discovered a new nitrogen-and phosphorus-containing polybasic acid in the extract of nuclei from human white blood cells. He called this material nuclein. At this time, there was a great interest in the chemical identity of constituents of living things, and a variety of chemists worked to characterise nuclein. Although Hartwig proposed in the 1870s[3] that nuclein was probably responsible for the transfer of genetic information, this was not proven until 1944, with the experiments of Avery and coworkers[4]. The final proof came with the elegant labelling experiments of Hershey and Chase in 1953[5] They showed that ^{32}P-labelled DNA was the genetic material in a bacteriophage, rather than ^{35}S-labelled protein.

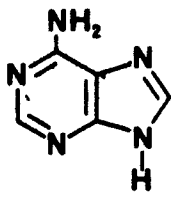
---

1. F. Miescher, *Hoppe-Seyler's Med. Chem. Unters*, 441, (1871).
2. J. E. Davies and H. G. Gassen, *Angew. Chem. Int. Ed. Eng.*, **22**, 13, (1983).
3. O. Hartwig, *Morphol. Jahrb.*, 1, (1875).
4. O. T. Avery, C. M. MacLeod, M. McCarty, *J. Exp. Med.*, **79**, 137, (1944).
5. A. D. Hershey, M. Chase, *J. Gen Physiol.*, 36, 39, (1953).

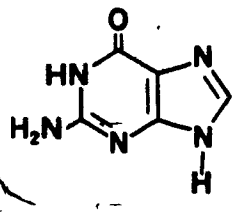
FIGURE 1. PRIMARY STRUCTURES OF DNA, RNA AND THE MOST COMMON HETEROCYCLIC BASES



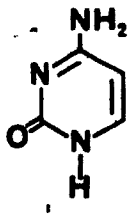
DNA R=H
RNA R=OH



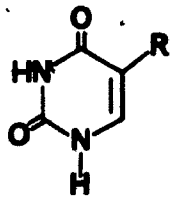
ADENINE



GUANINE



CYTOSINE



THYMINE R=CH_3
URACIL R=H

In the meantime, work was going on to identify the constituents of nucleic acids, a task not made easy by the size of the molecule and the opportunities for geometrical and stereoisomerism. By the beginning of the Great War, various groups had shown the presence of phosphoric acid, sugars and nitrogenous bases in the hydrolysed nucleic acid. In 1918, D-ribose was identified as a constituent, and the same group later identified 2'-deoxyribose as the sugar from other nucleic acids[6]. During the 1950s, the linkage scheme was identified as being 3'-5', and DNA, found mainly in the nucleus, and RNA, found mainly in the cytoplasm, were shown to be long linear polymers (see Fig. 1).

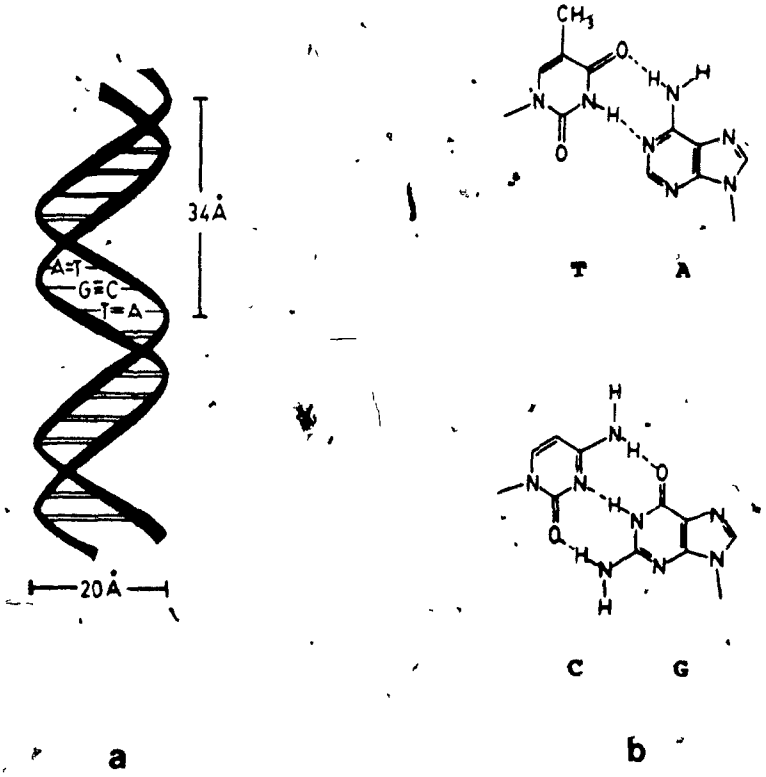


Fig. 2. (a) Double helix. (b) Hydrogen bonds between bases.

Watson and Crick, using X-ray crystal data provided by Wilkins and Franklin, proposed the now familiar double helix structure for DNA[7] (Fig. 2). In this structure, the helix is formed by two antiparallel chains of DNA which are intertwined in a double helix. The helix is right-handed and comprises ten base pairs per turn. The bases are turned inward and the duplex is stabilised by hydrogen bonds between the bases on opposite chains. Guanine (G) bonds with three H-bonds to

6. P. A. Levine, L. A. Mikeska, T. Mori, *J. Biol. Chem.*, 85, 785, (1930).
7. J. D. Watson and F. H. C. Crick, *Nature*, 171, 731, (1953).

cytosine (C), and adenine (A) with two to thymine (T). The phosphate diester backbone is turned to the outside. The particular helical form described by Watson and Crick is now known as B-DNA. Since this discovery, a number of variations on the theme have been observed, mainly modified in the number of bases per turn, the degree of hydration, or in the case of Z-DNA[8], the handedness of the helix.

As mentioned above, nucleic acids are the means by which genetic information is stored, transmitted and used. In other words, they are critical to the operation and construction of the cell, and, by extension, of the whole organism. This has made nucleic acids very interesting to scientists, as synthetic targets, and as objects of study to elucidate their role, and the exact mechanisms by which that role is carried out.

Deoxyribonucleic acids are largely found in the nucleus of the eukaryotic cell. In both prokaryotes (organisms whose cells do not contain a discrete nucleus) and eukaryotes (whose cells contain a nucleus wherein resides the DNA), the DNA sequence carries the actual genetic information, encoded in the order of the bases along the chain. DNA normally exists as a double helix, as described above. The transfer of the information to the remainder of the cell, and its translation into protein, are the province of ribonucleic acids. These exist in several forms. Messenger RNA (mRNA) is formed by enzymatic polymerisation of ribonucleotides using the DNA as a template. The mRNA, which is complementary to the DNA sequence of interest, migrates from the nucleus to the cytoplasm (for eukaryotes), and binds to the ribosome. This organelle, which also contains RNA (rRNA), is the site at which the information carried by the mRNA is translated into protein . Transfer RNAs (tRNA) exist in the cytoplasm, and their function is to transport the amino acids required for protein synthesis to the ribosome. These tRNAs have lengths in the 75-80 nucleotide range, making them challenging synthetic targets, long enough to demand excellent synthetic chemistry, yet discrete, independent units, which have a distinct function. Clearly, the central role of nucleic acids makes them of interest to the chemist, as well as to the biologist.

Synthesis of Nucleic Acids.

One of the more interesting developments in the field, from the chemists' point of view, has been the discovery of routes to the complete chemical synthesis of long strands of DNA and RNA. This allows the production of any desired sequence, mimicking natural sequences, making

8. A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, A. Rich, *Nature*, 282, 680, (1979).

any change, or making large quantities of a given sequence, free from contamination by other material.

Four routes to the synthesis of nucleic acids have been developed. In each case, the method was first applied to DNA, followed by RNA. This is due to the fact that, in the DNA case, there is no 2'-hydroxyl to protect (Fig. 1). The necessity of protecting this position in the case of RNA synthesis, coupled with the inevitable steric hindrance associated with such a protecting group, has led to a lag in RNA synthesis. This gap has recently been narrowed with the advent of highly selective protecting procedures, and the introduction of the very efficient phosphite triester procedure (*vide infra*).

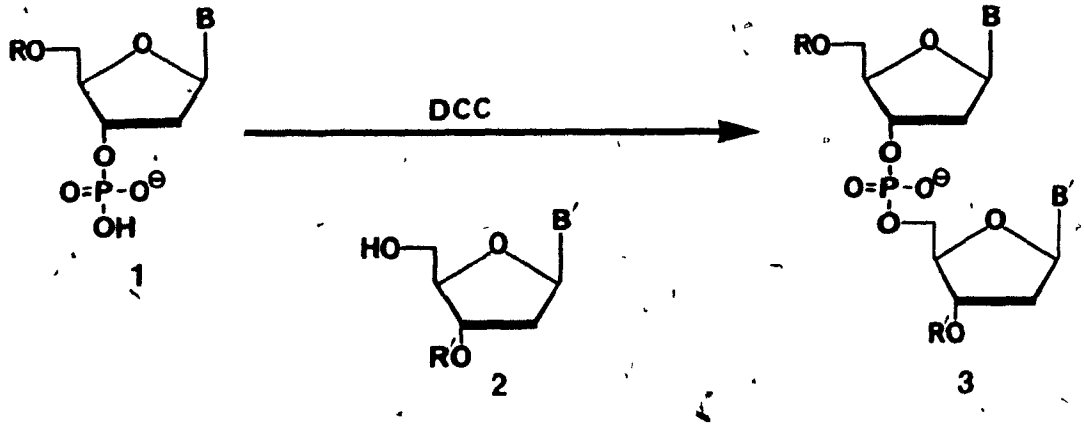
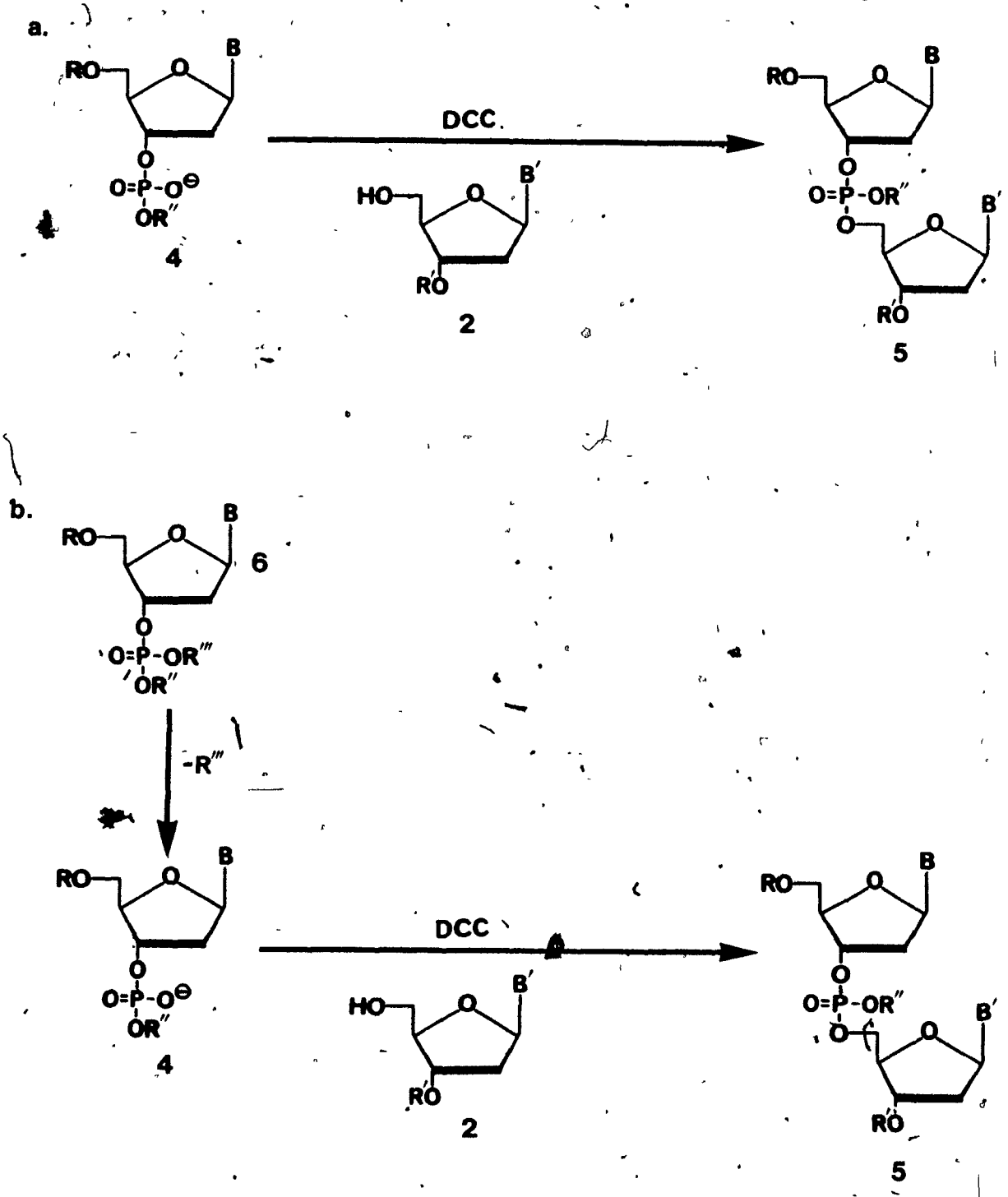


Fig. 3. Phosphate diester method.

The phosphate diester (Fig. 3) method was first introduced by Khorana[9] for DNA, and later by the same group, as well as that of Smrt and Sorm, for RNA[10]. In this procedure, a 3'-monoester **1** is activated with some appropriate condensing agent, such as dicyclohexylcarbodiimide, then reacted with the 5'-hydroxyl of a second nucleoside **2** to give a dinucleoside phosphodiester, **3**. This was the first commonly used synthetic route to nucleotides. However, coupling yields are relatively low, and purification of the product is difficult, due to the charged nature of the dinucleoside. The diester may also react with coupling reagents in subsequent steps.

9. P. T. Gilham, H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212, (1958).
10. (a) M. Smith, D. H. Rammler, I. H. Goldberg, H. G. Khorana, *ibid.*, **84**, 430, (1962). (b) J. Smrt, F. Sorm, *Coll. Czech. Chem. Comm.*, **27**, 73, (1962).

Fig. 4. (a) Phosphate triester method. (b) modified phosphate triester method.



The phosphate triester method (Fig. 4, a) was originally introduced by Todd[11] (the first reported chemical synthesis of a dinucleotide) and reintroduced by Letsinger[12] and Eckstein[13]. This method relies upon the activation of an *in situ* generated 3'-phosphodiester **4**, and subsequent reaction of this intermediate with the 5'-hydroxyl of the second nucleoside **2** to yield phosphotriester **5**. This uncharged material is easier to purify than the diester, and the yields are higher. In addition, the triester is stable, and not susceptible to further reaction. This method was subsequently improved by the use of differentially protected triesters (**6**), which could be made in advance and stored for long periods. One group could be removed *in situ* to yield **4**, followed by the usual coupling reaction. This procedure is referred to as the "modified" triester route (Fig. 4, b), and was introduced by Cramer[14] (DNA) and van Boom[15] (RNA). Good coupling yields are obtained, and since the triester starting material is prepared in advance and is quite stable, the method is amenable to routine use.

Even with these improvements, phosphate triester chemistry still suffers from relatively long coupling times, troublesome side products which make purification tedious, and yields, which, although acceptable for very short sequences, are too low for the efficient synthesis of very long molecules.

11. A. M. Michelson, A. R. Todd, *J. Chem. Soc.*, 2632, (1955).
12. R. L. Letsinger, K. K. Ogilvie, *J. Am. Chem. Soc.*, **89**, 4801, (1967).
13. F. Eckstein, I. Rizk, Angew. Chem., *Int. Ed. Eng.*, **6**, 949, (1967).
14. J. C. Catlin, F. Cramer, *J. Org. Chem.*, **38**, 245, (1973).
15. J. H. van Boom, P. M. J. Bergers, *Tetrahedron Lett.*, 4875, (1976).

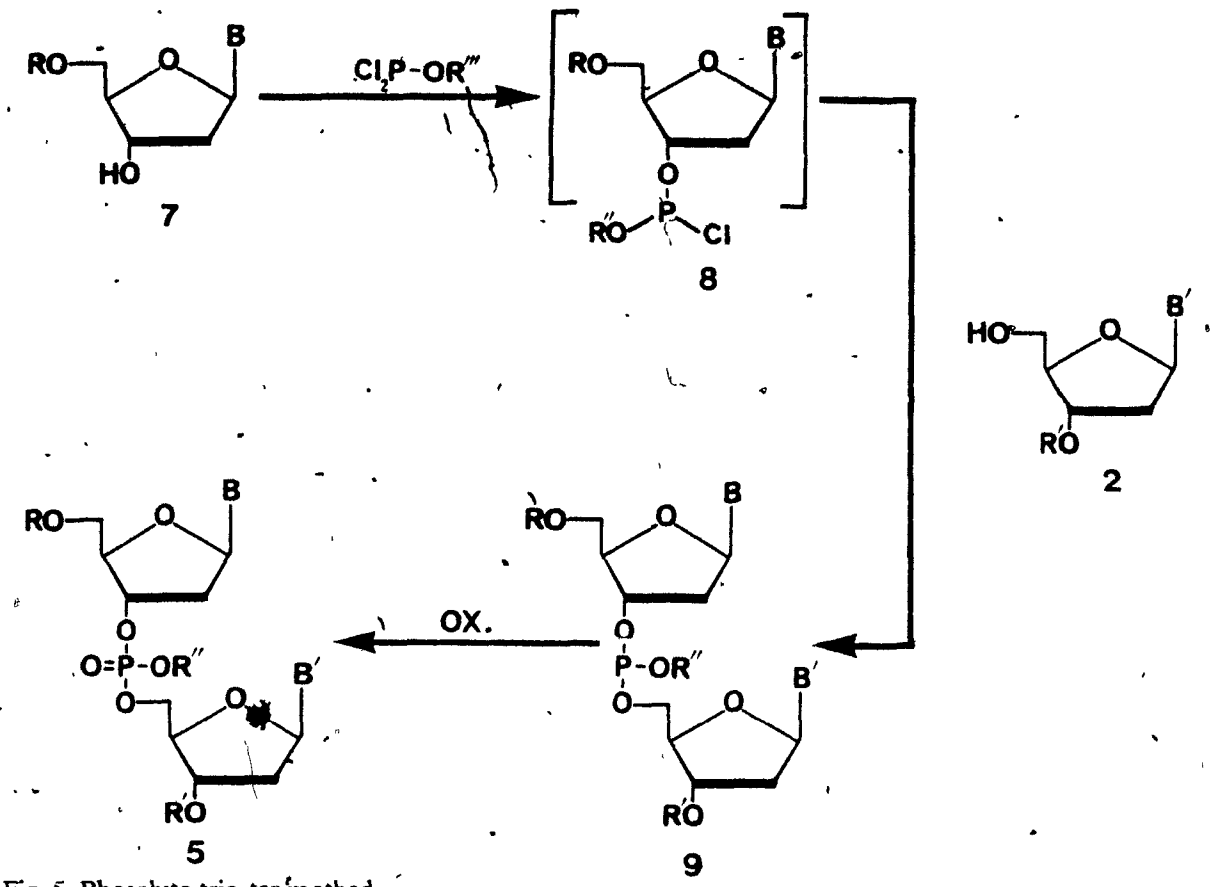


Fig. 5. Phosphite triester method.

The introduction of the phosphite triester method[16] (Fig. 5) for DNA synthesis, followed by its application to RNA[17], established the use of the more reactive trivalent phosphorus reagents. An appropriately 5'-protected nucleoside 7 is added to a solution of an alkoxyphosphodichlorodite in a solvent such as THF. The 3'-hydroxyl is phosphorylated to give intermediate 8 which is not isolated. Addition of a 3'-protected nucleoside allows the generation of the dinucleoside phosphite triester 9. This is oxidised *in situ* with iodine and water or *m*-chloroperbenzoic acid to yield the desired phosphate triester. The route is an advance over previous methods in that coupling yields are very high, and reaction times short. The replacement of one of the chlorine atoms of the dichloridite with a substituted amine allows the isolation of the reactive intermediate. This is the phosphoramidite route[18] (Fig. 6).

16. (a) R. L. Letsinger, J. L. Finnan, G. A. Heavner, W. S. Lunsford, *J. Am. Chem. Soc.*, **97**, 3278, (1975). (b) R. L. Letsinger, W. S. Lunsford, *ibid.*, **98**, 3655, (1976).
17. (a) K. K. Ogilvie, N. Theriault, K. L. Sadana, *ibid.*, **99**, 7741, (1977). (b) G. W. Daub, E. E. van Tamelen, *ibid.*, **99**, 3526, (1977).
18. S. L. Beaucage, M. H. Caruthers, *Tetrahedron Lett.*, **22**, 1859, (1981).

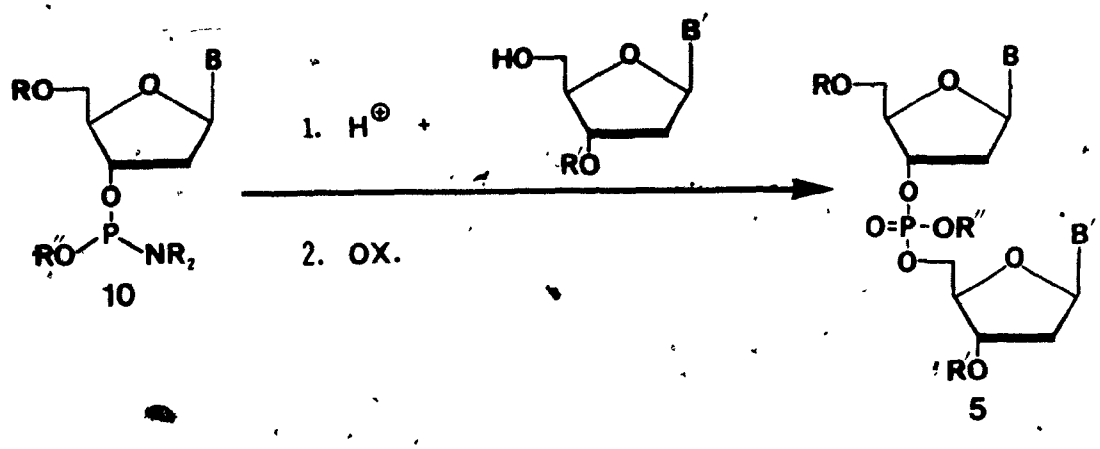


Fig. 6. Phosphoramidite method.

The 3'-hydroxyl of the protected nucleoside **7** is reacted with N, N-dialkylalkylphosphonamidic chloride, under basic conditions. The resulting nucleoside phosphoramidite **10** is usually isolated by precipitation from hexane. These compounds are stable to long term storage. The coupling reaction consists of activation of the amino function with an acid, frequently a tetrazole or a derivative, followed by coupling with the 5'-hydroxyl of the second nucleoside. Oxidation of the phosphite triester **9** yields the phosphate triester **5**. Coupling efficiencies are similar to those found using dichloridites. A variety of amino groups have been used. The original work by Beaucage and Caruthers employed dimethylaminophosphoramidites Others have used (for example) morpholino, diisopropylamino[19], and methylisopropylamino[20] groups.

The synthesis of long nucleotide sequences has been greatly enhanced by the use of solid-phase synthetic techniques. First introduced in the area of peptide chemistry by Merrifield[21], the process offers the advantages of high coupling yields, since large excesses of reagents may be used

19. L. J. McBride, M. H. Caruthers, *ibid.*, **24**, 245, (1983).
20. S. P. Adams, K. S. Kavka, E. J. Wykes, S. B. Holder, G. R. Gallupi, *J. Am. Chem. Soc.*, **105**, 661, (1983).
21. R. B. Merrifield, *Science*, **150**, 178, (1965).

and simply washed off the support; ease of automation; simplicity of purification, since the product is only purified after the last step; and small scale, preserving valuable and expensive materials. The technique has been used to good advantage in the nucleotide synthesis area for both DNA[22] and RNA[23].

Analogues of Nucleosides and Nucleotides.

Clearly, the chemical synthesis of naturally-occurring nucleic acids has been a stimulating and productive field. However, duplicating the work of nature has not been the chemists' only interest in this area. From Fig. 1, it is easy to see that there are a number of sites in the DNA or RNA molecule susceptible to chemical modification. For this reason, and because of the intimate link between these molecules and biological processes, modified bases, nucleosides (sugar plus base), and nucleotides (sugar, base and phosphate) have been extensively studied as chemotherapeutic agents, and as probes to study living systems.

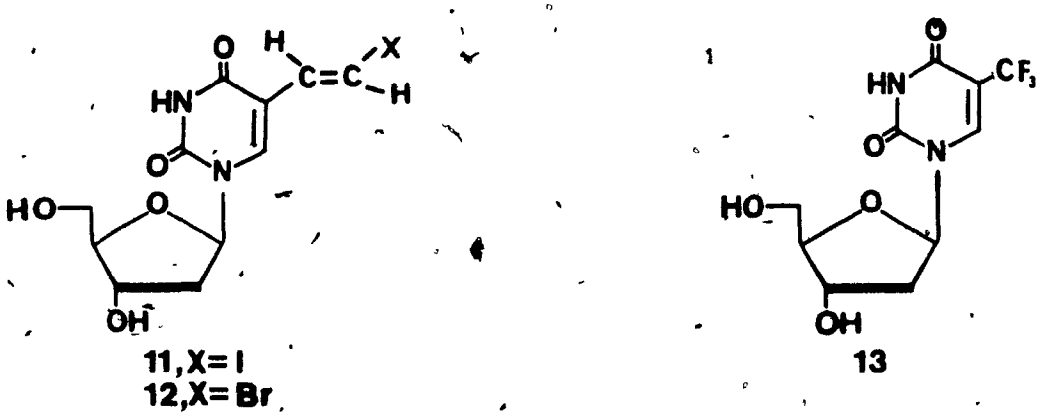


Fig. 7. Base-modified nucleosides.

22. *Oligonucleotide Synthesis - A Practical Approach*, M. J. Gait, ed., IRL Press, Oxford, (1984).
23. (a) K. K. Ogilvie, M. J. Nemer, *Tetrahedron Lett.*, 21, 4159, (1980). (b) R. T. Pon, K. K. Ogilvie, *ibid.*, 25, 713, (1984). (c) R. T. Pon, K. K. Ogilvie, *Nucleosides, Nucleotides*, 3, 485, (1984). (d) N. Usman, Ph. D. Thesis, McGill University, (1986).

The bases have been altered (Fig. 7 and below) by derivitisation at various positions, especially the 5-positions of uracil and thymine (11, 12, 13), deletion or insertion of various heteroatoms, or the construction of entirely novel ring systems. In addition, the point of attachment to the ring may be changed. Some of these molecules will be discussed later.

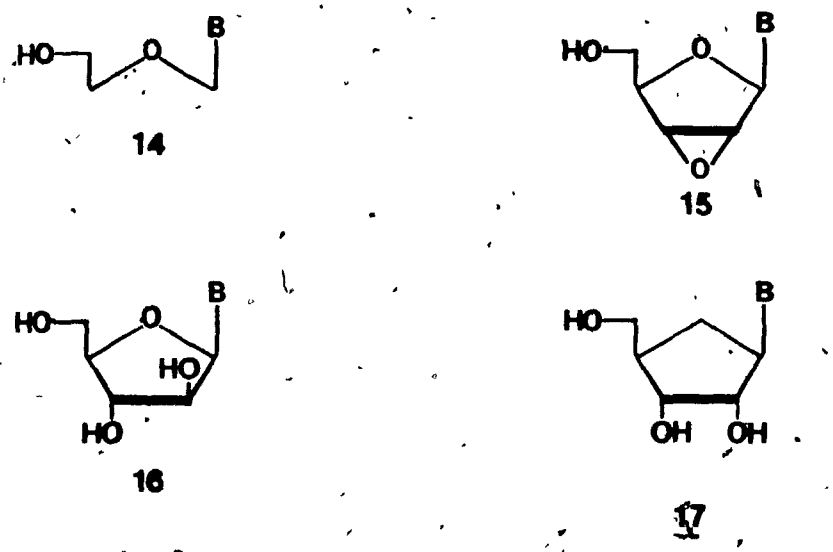


Fig. 8. Sugar-modified nucleosides.

Sugar rings are altered (Fig. 8) by addition or deletion of hydroxyls, their inversion (in the case of secondary hydroxyls) or their replacement by other atoms. The sugar ring itself may be opened up by deletion of one or more carbon atoms. The ether oxygen may be replaced by other heteroatoms or by carbon. The entire furanose sugar may be replaced by a different sugar, or the configuration at the anomeric position (β in the natural nucleosides) may be changed. Analogues may also provide for linkage between base and sugar at positions other than the anomeric, or the formation of additional links, as in the anhydronucleosides (*vide infra*).

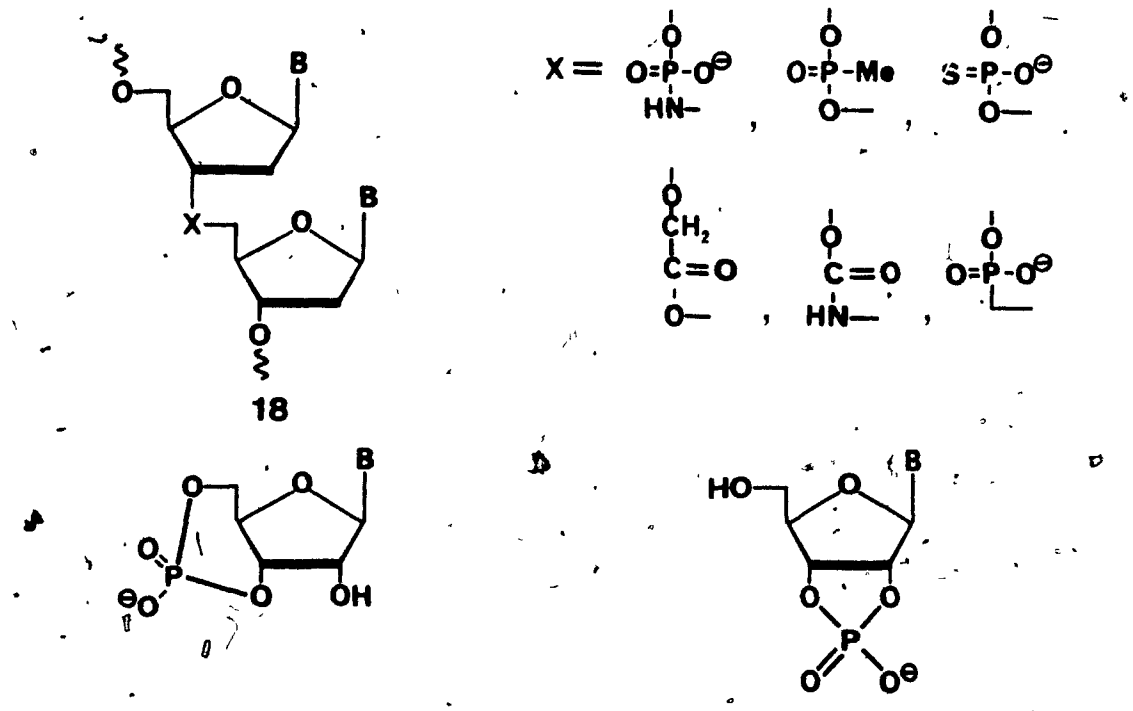


Fig. 9. Linkage-modified nucleotides.

In operating on the phosphate diester link, chemists have made changes (Fig. 9) in the position of the link, moving it to the 2'-position of the sugar, or forming 2'-3'- or 3'-5'-cyclic nucleotides. Phosphate diesters have been replaced with a variety of groups, carbonate, carbamate, phosphonate, phosphorothioate and other links having been introduced.

Uses of analogues.

The past fifty years have seen a wealth of agents brought forth to combat disease. Most of these have been in the field of antibiotics. From the early sulfa drugs, through the development of penicillin, to today's broad spectrum antibiotics, we have had great success in controlling bacterial infection. More modestly, we have also been able to bring under control many parasitic infections. However, effective treatment of many viral illnesses, ranging from the common cold to acquired immune deficiency syndrome (AIDS), has escaped us. Similarly, many types of cancers are still resistant to treatment. The reason for this contrast is to be found in the nature of the illness. Bacterial infections are caused by agents whose biochemistry is radically different from that of the host. Most antibiotics take advantage of these differences. For example, the β-lactam antibiotics interfere with the construction of the bacterial cell wall, a process which does not exist in animal

cells. Thus we can attack such bacterial infections with relative impunity from the point of view of the patient.

Unfortunately, the same condition does not obtain in the case of viral infections[24]. Outside the host cell, viruses are essentially metabolically inert. Thus they are not susceptible to attack by most types of drugs. Once the virus enters the target cell, it largely takes over the machinery of the host. Targets for drug action are limited due to the need to avoid toxicity to the host. This problem has led to great difficulties in the design of effective antiviral agents.

The same problem arises in the attempt to treat cancers. Attacking the cancerous cell is often hazardous in that its biochemistry is very similar to that of the normal cell, and often it is necessary to rely on methods such as surgery or radiation therapy. These procedures are often dangerous or even life-threatening in themselves. Chemotherapy is time-consuming, and usually has side effects which are at least unpleasant, and often dangerous. The drugs used are generally very toxic, and resistance is frequently encountered. Since nucleic acids are so fundamental to the growth and reproduction of cells, many nucleoside and nucleotide analogues have been explored, with some success, as antitumor agents, in the hope that they will be more toxic to the tumor cell than to the host. The search for effective antitumor and antiviral agents based on analogues of nucleic acid components has been reviewed extensively in recent years[25]. Some analogues have been found to be effective as chemotherapeutic agents. Examples (Fig. 10) are FIAC (**19**), acyclovir (**20**), arabinocytidine (**22**), and arabinoadenosine (**23**). BIOLF-62 (**21**, DHPG, NDG) is effective against herpesviruses.

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24. M. S. Hirsch, J. C. Kaplan, *Scientific American*, 76, (May, 1987).
25. (a) E. de Clercq, *Chem. Scripta*, **26**, 41, (1985). (b) E. de Clercq, *Nucleosides, Nucleotides*, **4**, 3, (1985). (c) R. Dolin, *Science*, **227**, 1296, (1985). (d) B. Oberg, N.-G. Johansson, *J. Antimicrob. Chemother.*, **14** (Suppl. A), 5, (1984). (e) R. T. Walker, *ibid.*, **14** (Suppl. A), 119, (1984).(f) R. Datema, N.-G. Johansson, B. Oberg, *Chem. Scripta*, **26**, 49, (1985). (g) A. Holy, *ibid.*, **26**, 83, (1985). (h) J. A. Montgomery, *Acc. Chem. Res.*, **19**, 293, (1986). (i) R. K. Robins, *Chem. Eng. News*, Jan. 27, (1986), p. 28.

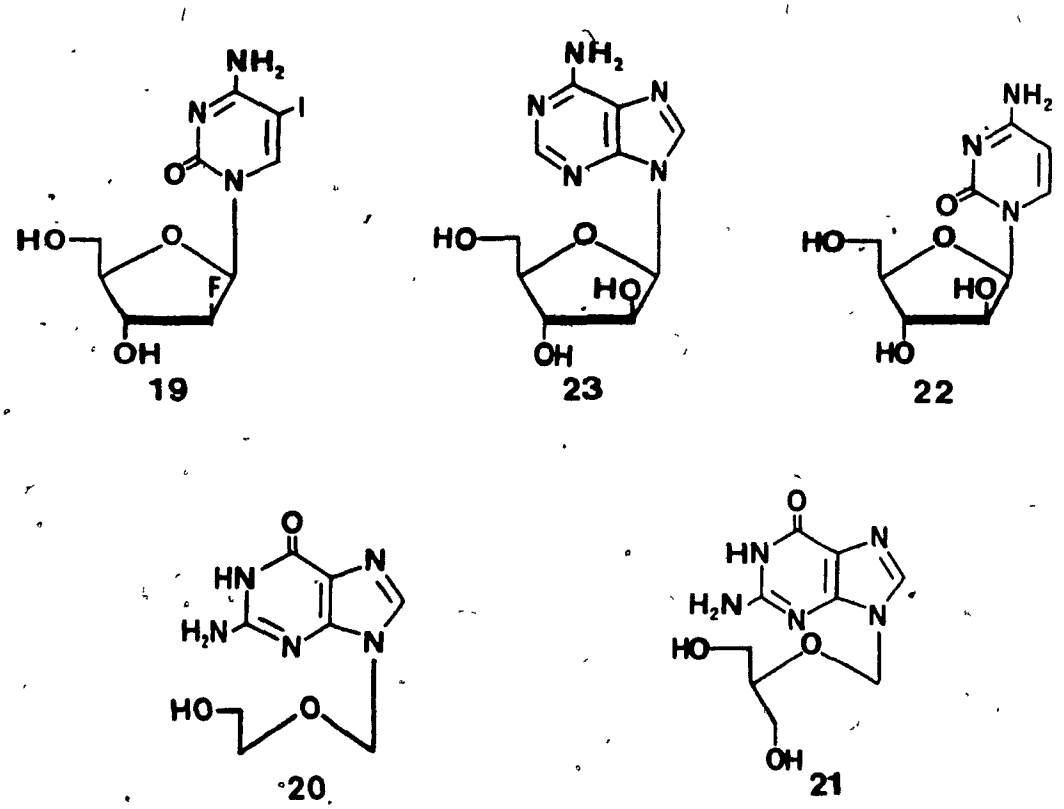

Fig. 10. Some nucleoside analogues with chemotherapeutic activity.

Other analogues are synthesised as probes of enzyme active sites. This is especially true of the phosphorothioate analogues, which have been used to determine the stereochemistry of the active sites of various phosphodiesterases. In other cases, fluorescent base-modified nucleosides have been incorporated into nucleotide sequences as reporter sites, to determine the biochemical fate of the sequence. This is particularly useful in hybridisation studies, where fluorescence tags may be used instead of radiolabels. It is not the purpose of this introduction to give an exhaustive description of the different types of analogues which have been reported. Rather, a short review of the sugar-modified nucleosides, and phosphate-modified nucleotides will be presented. A review of the general area has recently been published[26].

The work to be described in this thesis is divided into two parts. In the first, a method for selectively synthesising arabino- and xylonucleosides involving all four common bases will be described. In the second the synthesis and characterisation of a novel nonionic nucleotide analogue will be reported, along with some physical studies. Thus, at this point, the field of sugar-modified nucleosides will be described.

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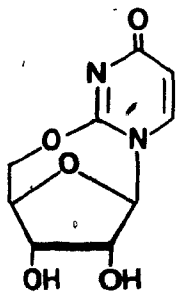
26. *Nucleosides, Nucleotides, and their Biological Applications*, J. L. Rideout, D. W. Henry, L. M. Beacham III, eds., Proc. 5th Int. Round Table, Academic Press, N. Y., N. Y., (1983).

Sugar-modified nucleosides.

A wide variety of sugar-modified nucleosides have been reported in the literature. These may be broadly categorised as halosugar nucleosides, C-nucleosides, nucleosides involving sugars other than ribose or 2-deoxyribose, anhydro- or cyclonucleosides, and some miscellaneous analogues.

Halosugar nucleosides are generally synthesised by replacement of one of the hydroxyls of the native nucleoside with the desired halogen. Usually this is accomplished by converting the hydroxyl to a good leaving group, followed by nucleophilic displacement by halogen. In this type of approach, care must be taken to avoid involvement of the base to form an anhydronucleoside, although this reaction may be used as part of some syntheses. 2,5'-, 2,3'-, and 2,2'-anhydronucleosides may form (**24**, **25**, and **26**, respectively).



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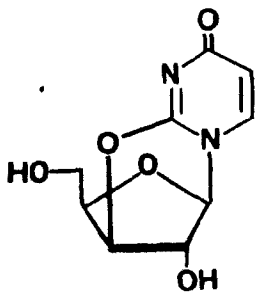

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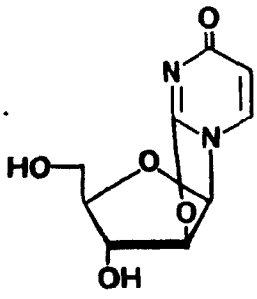
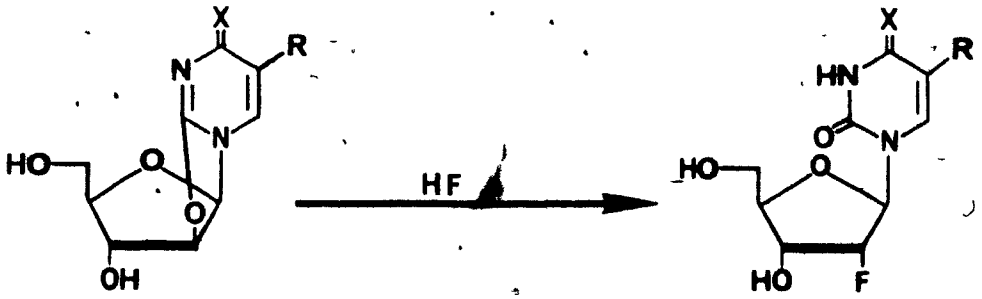


26

In the 1960s, Fox and coworkers[27] reported a series of studies in which 2'-deoxy-2'-fluoro derivatives of uridine, 5-fluorouridine and cytidine (**28**, X=O or NH, R=H or F) were synthesised by treatment of the 2,2'-anhydronucleosides (**27**) with hydrogen fluoride (Scheme 1). The stereochemical course of this reaction gives the halogen substitution in the same configuration as the original hydroxyl. The same group produced 9-(2-deoxy-2-fluoro-α and β-D-arabinofuranosyl) adenines by fusion of the fluorosugar with 2,6-dichloropurine. The anomeric mixture was separated, and the base converted to adenine by ammonolysis of the 6-chloro substituent, followed

27. (a) J. F. Codington, I. L. Doerr, J. J. Fox, *J. Org. Chem.*, **29**, 558, (1964). (b) I. L. Doerr, J. J. Fox, *ibid.*, **32**, 1462, (1967). (c) J. F. Codington, I. L. Doerr, J. J. Fox, *Carbohyd. Res.*, **1**, 455, (1966).

hydrogenolysis of the 2-chloro group[28]. This same laboratory has also reported the synthesis of several other fluorosugar-nucleosides[29].

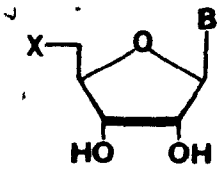


27 28

Scheme 1.

Verheyden and Moffatt[30] used methyltriphenoxyphosphonium iodide to replace the 5'-hydroxyl of various nucleosides to give the 5'-iodo-5'-deoxynucleosides (29, X = I). In some cases, the unprotected nucleoside could be used with good selectivity, while in others, the 2',3'-O-isopropylidene derivative gave good results. These workers also found that considerable amounts of anhydronucleoside (30), the product of reaction between the heterocyclic base and the 5'-hydroxyl of the phosphonylated intermediate, were formed under the basic conditions of the reaction. These workers also found[31] that the Mitsonobu reaction of carbon tetrabromide or tetrachloride, triphenylphosphine and the 5'-hydroxyl groups of some 2', 3'-protected nucleosides gave the 5'-halo-5'-deoxynucleosides 29 in good yield. The same reaction with no secondary hydroxyl protection led to slightly reduced, but still quite respectable, yields of the same product. Protection of the primary hydroxyl led to quite selective reaction at the 2'-position of ribonucleosides, with bromination proceeding with retention (32) and chlorination with inversion(31).

28. J. A. Wright, N. F. Taylor, J. J. Fox, *J. Org. Chem.*, 34, 2633, (1969).
29. J. A. Wright, D. P. Wilson, J. J. Fox, *J. Med. Chem.*, 13, 269, (1970).
30. J. P. H. Verheyden, J. G. Moffatt, *J. Org. Chem.*, 35, 2319, (1970).
31. J. P. H. Verheyden, J. G. Moffatt, *ibid.*, 37, 2289, (1972).



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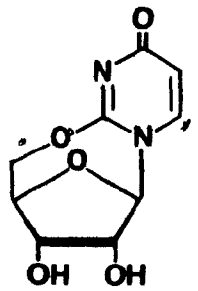

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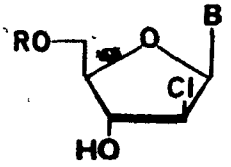

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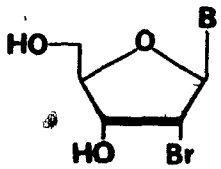
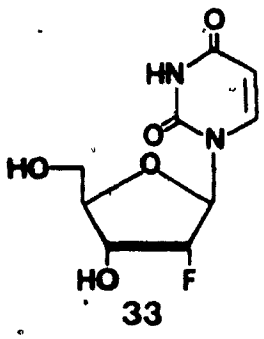


32

A German group synthesised 3'-deoxy-3'-fluorouridine (**33**) by building the heterocyclic ring directly onto 5-O-benzyl-3-deoxy-3-fluoroarabinose[32]. This reaction sequence proceeds in low yield but provides an unequivocal route to the desired compound. This same group also reported, in the same publication, a higher yield route based on the anhydrous hydrogen fluoride cleavage of 2,3'-anhydro-1-(β-D-xylofuranosyl)uracil **25**. This yields a mixture of the 3'-substituted and 2'-substituted products **33** and **34** in a 2:3 ratio. The authors speculated that the latter product is the result of the rearrangement of the anhydro link from the 3'- to the 2'-position, followed by HF cleavage. This group has also reported the synthesis of several other fluorodeoxynucleosides in the pyrimidine series[33].

32. G. Kowollik, K. Gaertner, P. Langen, J.-Carbohyd., *Nucleosides & Nucleotides*, **2**, 191, (1975).
33. (a) G. Etzold, R. Hintsche, G. Kowollik, P. Langen, *Tetrahedron*, **27**, 2463, (1971). (b) P. Langen, G. Etzold, G. Kowollik, *Acta Biol. Med. Ger.*, **28**, K 5, (1972). (c) P. Langen, G. Kowollik, G. Etzold, H. Venner, H. Reinert, *ibid.*, **29**, 483, (1972).



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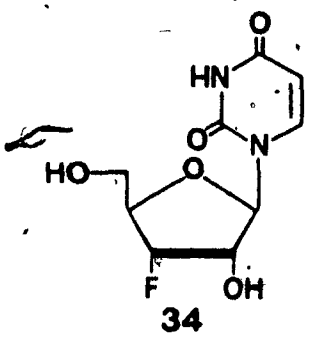
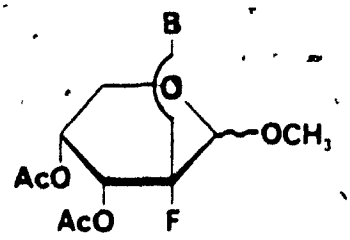


34

An and Bobek[34] developed a route, based on their own gem-difluorosugar synthesis (ref. 1 of their paper), to 2'-fluoro-2'-deoxyuridine analogues (35) with the heterocyclic base attached at the 2'-position of the sugar (in this case a pyranose). Methyl 3,4-di-O-acetyl-2-deoxy-2,2-difluoro-D-erythro-pentopyranoside was treated with HBr in methylene chloride to give the 2-bromo-2-fluorosugar. This was coupled with trimethylsilylated uracil in the presence of mercuric oxide-mercuric bromide to give exclusively the base in the arabino configuration. The α and β anomers were separated and the stereochemistry assigned on the basis of the proton coupling constants. These molecules showed antitumor activity in leukemic mice.

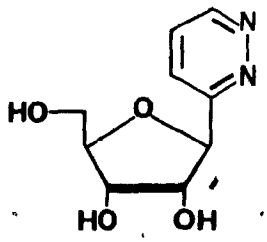


35

Another point at which chemists have modified nucleoside structure is at the attachment of the base to the sugar. Normally, the base is attached via a nitrogen atom, typically N-9 in

34. S.-H. An, M. Bobek, *Tetrahedron Lett.*, 27, 3219 (1986).

purines and N-1 in the pyrimidjnes. However, a few rare natural nucleosides, such as pseudouridine, have a carbon-carbon glycosidic bond. Recently, a number of synthetic C-nucleosides have been investigated as potential therapeutic agents.



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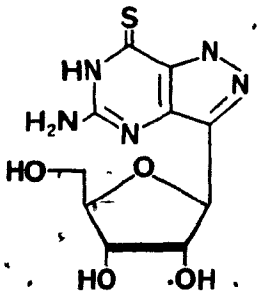
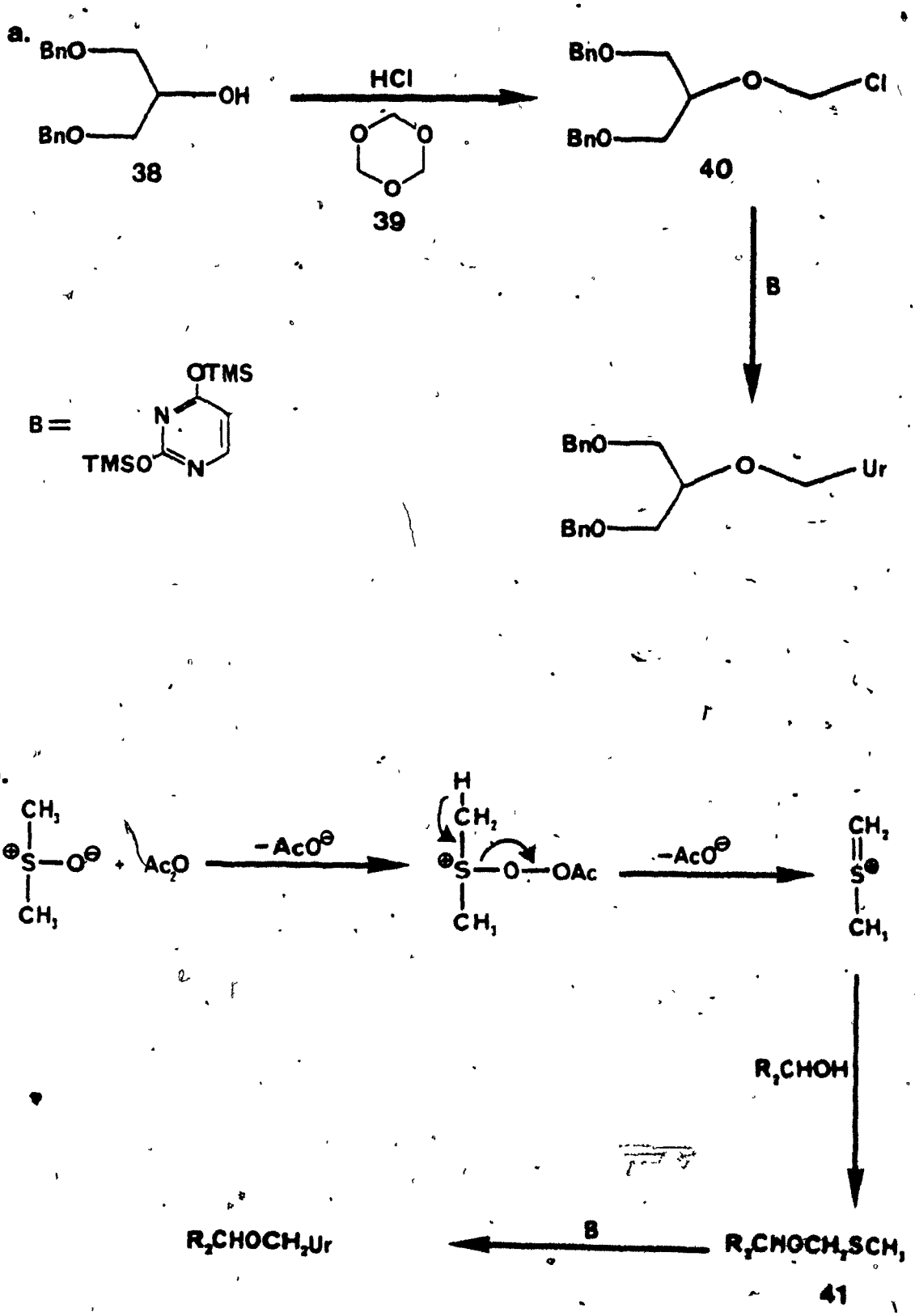


37

For example, Furukawa *et al.*[35] synthesised a series of 3-(β-D-ribofuranosyl)pyridazines (*e.g.* **36**) by reaction of 2,3,5,-protected ribosyl bromide with 2-(chloromercuri)furan to give an anomeric mixture of the ribosyl furanoside. Acid treatment epimerised the α to the β anomer in good yield. The furan ring was then converted to the pyridazine via bromine-methanol oxidation, followed by treatment with hydrazine under acidic conditions. Acton and Ryan[36] noted that the therapeutic potential of thioguanosine was defeated by its biological cleavage to thioguanine, which is more toxic. They thus synthesised the related C-nucleoside, 3-glycofuranosyl-5-amino-pyrazole[4,3-d]pyrimidine-7-thione (**37**), which is similar to the previously known formycins. No biological results were reported.

In 1978, Schaeffer and coworkers[37] reported the synthesis and antiviral activity of a number of nucleoside analogues (**14**) in which the sugar was replaced with a 2-hydroxyethoxymethyl group. The most active compound contained guanine as the base. This compound, known as acyclovir, is one of the few effective antiviral agents currently in clinical use, and is especially effective against the herpes group of viruses. The chloromethylether (*vide infra*)of the side chain was coupled with 2,6-dichloropurine. The 6-chloro function was then displaced with ammonia in methanol, followed by nitrous acid deamination. Ammonolysis of the 2-chloro group gave the desired guanine derivative. This was the first reported acyclonucleoside having significant

35. I. Maeba, K. Iwata, F. Usami, H. Furukawa, *J. Org. Chem.*, **48**, 2998 (1983).
36. E. M. Acton, K. J. Ryan, *J. Org. Chem.*, **49**, 528 (1984).
37. H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, P. Collins, *Nature*, **272**, 583 (1978).


a.
BnO
OH
BnO
38
HCl
39
BnO
O
Cl
BnO
40
B
OTMS
B=
N
TMSO
N
BnO
O
Ur
BnO
b.
CH3
S
O
CH3
Ac2O
-AcO⊖
H
CH2
S
O
OAc
CH3
-AcO⊖
CH2
S
CH3
R2CHOH
R2CHOCH2Ur
B
R2CHOCH2SCH3
41

Scheme 2.

antiviral activity. Subsequently, a variety of groups began publishing reports of a very wide variety of acyclic analogues. Ogilvie *et al.*[38] reported even greater activity for another analogue, 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]-methyl]guanine (BIOLF-62, **21**), which bears two primary hydroxyls. It may be pictured as guanosine with the 2'-carbon removed. The activity of this compound was subsequently reported by other groups[39]. This pair of findings has stirred a great interest in the synthesis of similar analogues[40] which vary either in the nature of the heterocyclic base, or the acyclic side chain. These later compounds have not exhibited the potent antiviral activity of BIOLF-62 and acyclovir.

Most of these syntheses are based on the chloromethyl or the related methyl thiomethylether route for creating the side chain and attaching it to the base (Scheme 2a). For example, in the case of BIOLF-62, 1,3-di-O-benzylglycerol (**38**) is treated with anhydrous hydrogen chloride and paraformaldehyde (**39**) to yield chloromethyl ether **40**, which is then coupled with the persilylated base. Alternatively (Scheme 2b), the protected glycerol is treated with dimethylsulphoxide in acetic acid/acetic anhydride to yield the methyl thiomethylether (**41**), which is then coupled to the base. Deprotection yields the final product Other routes have also been used. (S)-9-(2,3-dihydroxy-1-propoxymethyl)guanine was synthesised[41] in enantiomerically pure form by coupling the base to the chloromethyl ether of methyl 2,3,4,-tri-O-benzyl- -D-glucopyranoside, followed by debenzylation, sodium periodate oxidation and sodium borohydride reduction. Acid hydrolysis of the resulting hemiacetal gave the desired product. Periodate cleavage followed by borohydride reduction was also applied to the synthesis of a series of trihydroxynucleosides, this time with the reagents supported on resins[42]. This route is used to cleave the 2'-3' carbon-carbon bond in all four common ribonucleosides, followed by *in situ* reduction of the dialdehyde product (**42**), to give the desired trihydroxy compound (**43**, Scheme 3)

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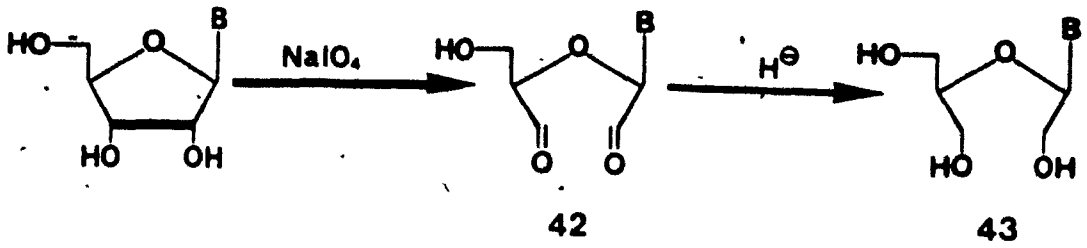
38. K. O. Smith, K. S. Galloway, W. L. Kennell, K. K. Ogilvie, B. K. Radatus, *Antimicrob. Agents Chemother.*, **22**, 55 (1982).
39. (a) W. T. Ashton, J. D. Karkas, A. K. Field, R. L. Tolman, *Biochem. Biophys. Res. Comm.*, **108**, 1716 (1982). (b) J. C. Martin, C. A. Dvorak, D. F. Smee, T. R. Matthews, J. P. H. Verheyden, *J. Med. Chem.*, **26**, 759 (1983)
40. (a) H. J. Schaeffer, *Nucleosides, Nucleotides and their Biological Applications*, J. L. Rideout, D. W. Henry, L. M. Beacham III, eds, Academic Press, N.Y., N.Y. (1983), 1, and refs. therein. (b) M. A. Tippie, J. C. Martin, D. F. Smee, T. R. Matthews, J. P. H. Verheyden, *Nucleosides & Nucleotides*, **3**, 525 (1984). (c) M. J. Robins, P. W. Hatfield, J. Balzarini, E. De Clercq, *J Med. Chem.*, **27**, 1486 (1984). (d) K. K. Ogilvie, H. R. Hanna, N. Nguyen-ba, K. O. Smith, *Nucleotides & Nucleotides*, **4**, 507 (1985), and refs. therein. (e) S. Boisvert, Ph. D. Thesis, McGill University (1986).
41. M. MacCoss, A. Chen, R. L. Tolman, *Tetrahedron Lett.*, **26**, 1815 (1985).
42. M. Bessodes, K. Antonakis, *Tetrahedron Lett.*, **26**, 1305 (1985).



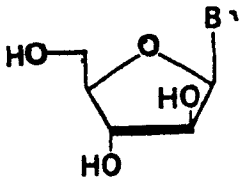
42 43

Scheme 3.

This oxidation was first applied to nucleosides by Todd[43] in a study to determine the structure of some synthetic nucleosides. The reduction of the dialdehyde with borohydride was introduced by Lerner[44] for the synthesis of trihydroxy nucleosides. More recently, this route has been examined by de Clercq[45] as a means of preparing a range of acyclouridine derivatives. Some groups have incorporated these acyclic nucleosides into nucleotide sequences[46]

One area heavily examined in the past three decades has been the synthesis of nucleosides containing sugars whose hydroxyls have unnatural configurations. In particular, modifications involving the selective inversion of one or more hydroxyls to create arabino-, xylo-,or lyxonucleosides (44, 45, 46) have been extensively investigated. Arabinonucleosides have been used as antitumor and antiviral agents. The synthesis of these molecules is not trivial, since there is the problem of selective protection of hydroxyls, in order to perform reactions at only the desired position (2' or 3' for ribonucleosides, 3' for deoxyribonucleosides). Furthermore, involvement with the base (anhydronucleoside formation) is a common difficulty Part of the research described in this thesis was directed toward development of a simple and broadly applicable route to xylo- and arabinonucleosides.

43. A. Todd, *J. Chem. Soc.*, 592 (1944).
44. L. M. Lerner, *Carbohydr. Res.*, 13, 465 (1970).
45. A. S. Jones, M. J. McClean, H. Tanaka, R. T. Walker, J. Balzarini, E. de Clercq, *Tetrahedron*, 41, 5965 (1985).
46. (a) S. N. Mikhailov, W. Pfleiderer, *Tetrahedron Lett.*, 26, 2059 (1985). (b) C. Juby, M. Sc. Thesis, McGill University (1986)



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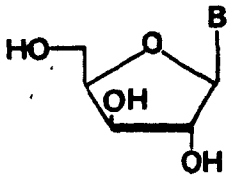

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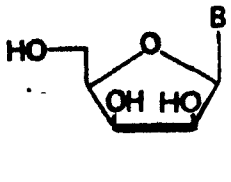
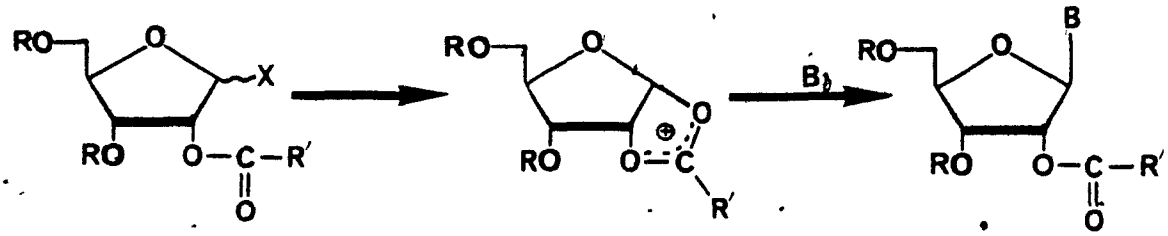


46

Methods of synthesising these compounds may be divided into two groups. First, a suitably protected sugar with the desired stereochemistry may be fused with the nucleoside base to give the desired product. The use of participating protecting groups at the 2-position of the sugar may ensure a *trans* relationship between the 1- and 2-substituents if the reaction follows Baker's *trans* rule[47].



47 48 49

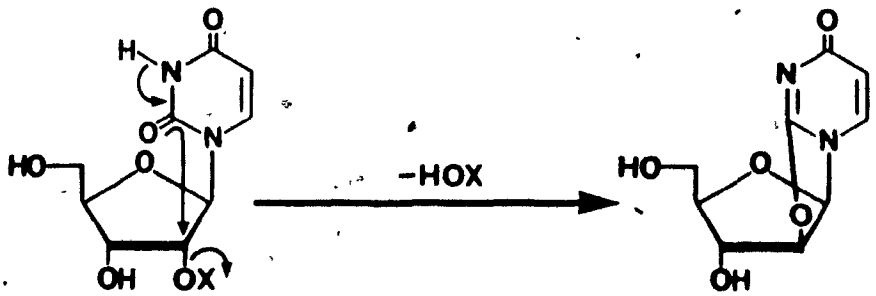
Scheme 4. Baker's *trans* rule.

Baker's rule states that, if an acyl protecting group (usually acetyl) is present at the 2-position of the sugar (47), the product of glycosidic bond formation will be *trans* to the 2-hydroxyl. In other words, the fusion reaction between a nucleoside base and a sugar with a participating group

47. (a) B. R. Baker, J. P. Joseph, R. E. Schaub, J. H. Williams, *J. Org. Chem.*, 18, 1786 (1954). (b) B. R. Baker, *Ciba Found. Symp. Chem. Biol. Purines*, G. E. W. Wolstenholme, C. M. O'Connor, eds., Little, Brown & Co. Boston (1957), p. 120.

participating group at the 2-position will yield a nucleoside with a *trans* relationship between the base and the 2'-hydroxyl. This may be useful for the synthesis of ribo- or xylonucleosides, but interferes with the synthesis of aranucleosides, which display a *cis* relationship between the base and 2'-hydroxyl. The stereospecifity of glycosylations which follow the rule is thought to be due to the intermediacy of an orthoester (48, Scheme 4). The use of nonparticipating protection, such as benzyl ethers, almost inevitably yields the anomeric mixture with respect to the glycosidic bond, with the purification problems associated with this type of mixture. Indeed, the α anomer may be favoured due to the anomeric effect. Usually the natural β anomer is desired

Alternatively, one may take the naturally occurring ribonucleoside or deoxyribonucleoside and operate on it to invert the stereochemistry at the desired position. This usually involves some form of selective protection, which has often been difficult to achieve. The usual approaches to inversion at a secondary carbon bearing a hydroxyl group may not be effective, since conversion to a good leaving group, followed by Sn2 displacement by water, for example, may invite the formation of anhydronucleosides (see Scheme 5) Until the start of our work, there was no general method in the literature for the synthesis of xylo- and arabinonucleosides.



50 26

Scheme 5.

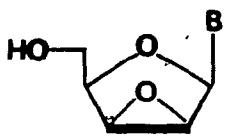
The synthesis of ara- and xylonucleosides goes back more than three decades. In the late 1940s and early 1950s, Lythgoe published a series of papers[48] in which xylonucleosides of adenine (45, B=adenine) and theophylline were described. The fully acetylated xylosylchloride was coupled

48. (a) N. W. Bristow, B. Lythgoe, *J. Chem. Soc.*, 2306 (1949). (b) P. Chang, B. Lythgoe, *ibid.*, 1992 (1950).

with the base (as its silver salt) to yield only the β form of the nucleoside. The route required the tedious, multistep synthesis of the protected sugar, and the final product was obtained in only moderate (40-50%) yield.

Baker and Schaub[49] started from 2,3,5-tri-O-benzoylxylofuranosyl-chloride, followed by fusion with a modified adenine, and a long series of synthetic manipulations, to arrive at the arabino analogue of puromycin. Baker also synthesised[50] xylo-A (**45**, B=adenine) from 2,3,5-tri-O-benzoylxyfuranosyl bromide by coupling with the N-protected base in the presence of mercury salts. The desired product was recovered in moderate yield.

In a study on the synthesis of ribonucleosides by the fusion method, Fox *et al.*[51] fused the mercury derivative of N-acetyl cytosine with tri-O-benzoyl-D-xylofuranosyl chloride to make, after deprotection, 1-β-D- xylofuranosylcytidine (xylo-C, **45**, B=cytosine) in good yield. A synthesis of ara-A (**23**) was reported[52] which was based on the formation of the 2',3'-anhydronucleoside (**51**) derived from xylo-A (**45**, B=adenine) by displacement of the 2'-mesylate. This epoxide was then opened by heating with sodium benzoate in moist DMF to yield predominantly the arabino nucleoside. This product was contaminated with a small amount of the xylonucleoside. Interestingly, no opening of the epoxide occurred when the solvent was changed to diglyme. The authors postulated that a complex is formed between the DMF and the benzoate ion which increased the ion's nucleophilicity.



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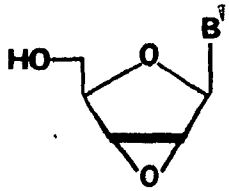


52

Yung and Fox[53] reported the synthesis of xylo-U (**45**, B=uracil) by the mesylation of 2',5'-di-O-trityluridine, followed by sodium benzoate-DMF-water treatment to give the 2,3'-

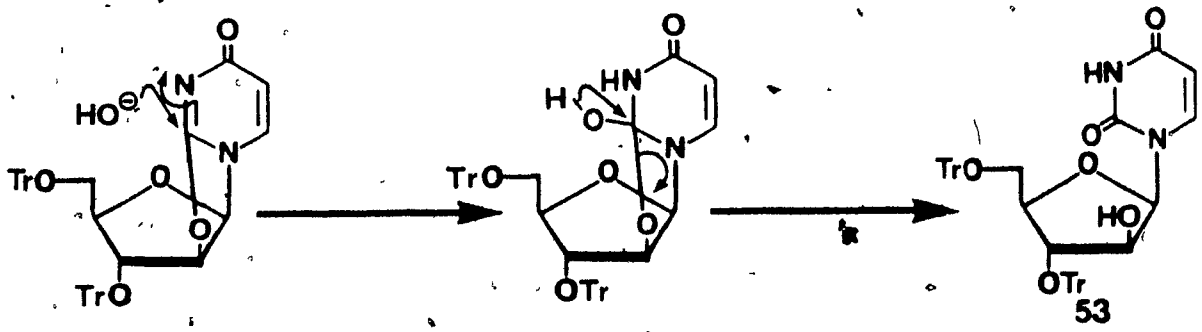
49. B. R. Baker, R. E. Schaub, *J. Am. Chem. Soc.*, 77, 5900 (1955).
50. B. R. Baker, K. Hewson, *J. Chem. Soc.*, 966 (1957).
51. J. J. Fox, N. Yung, I Wempen, I. L. Doerr, *J. Am. Chem. Soc.*, 79, 5060 (1957).
52. (a) W. W. Lee, A. Benitez, L. Goodman, B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2648 (1960). (b) E. J. Reist, A. Benitez, L. Goodman, B. R. Baker, W. L. Lee, *J. Org. Chem.*, **27**, 3274 (1962).
53. N. C. Yung, J. J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961).

anhydronucleoside (25). Treatment with aqueous base, followed by detritylation, led to xylo-U. The 3',5'-isopropylidene derivative of this molecule could be mesylated, and treated with base to form the 2,2'-anhydronucleoside (26), which could be opened to yield lyxo-U (46, B = uracil).

Many of the syntheses involving fusion of a base with the tetraacetylated sugar tend to give only the β-anomer if the 2'-hydroxyl is in the "down" configuration, and this has been postulated (*vide supra*) to be due to the intermediacy of an oxocarbonium ion. A *caveat* was provided in 1963[54] by Lee *et al.* This group found that fusion of various pyrimidines with tetra-O-acetyl xylose gave anomeric mixtures of the nucleosides. They proposed that the xylose configuration reduced the steric hindrance of the "bottom" side of the sugar ring, compared to the ribose configuration, allowing approach by the incoming heterocyclic base from either side.

The use of nonparticipating protecting groups was explored by Glaudemans and Fletcher[55] who used 2,3,5,-tri-O-benzylarabinofuranosyl chloride to couple to N-benzoyl adenine in the presence of molecular sieves. The β-anomer of ara-A (23) was the major product, isolated in 46% yield. Some α-anomer was also isolated.

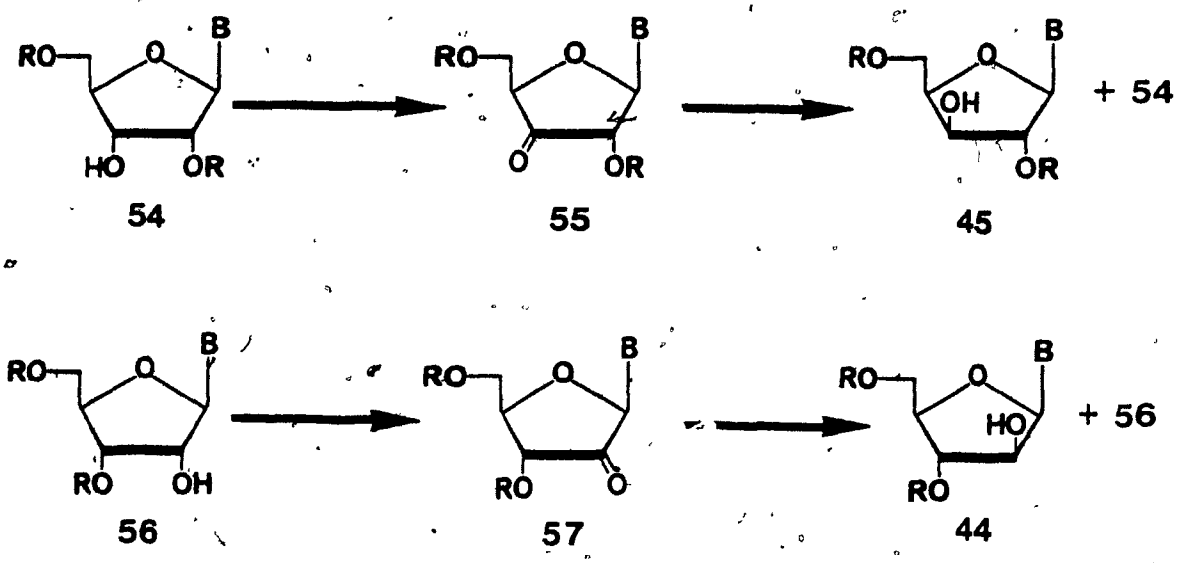
In a successful attempt to prove the structure of some 2'-halogenated uridines, Fox *et al.*[56] made the 3',5'-di-O-trityl derivative of the supposed 2'-halogeno-2'-deoxyribonucleosides and treated them with base. The product was dityrityl ara-U (53), produced by 2,2'-anhydronucleoside formation, followed by hydrolysis of the anhydro bridge (Scheme 6).



Scheme 6.

54. W. W. Lee, A. P. Martinez, G. L. Long, L. Goodman, *Chem. Ind. (London)*, 2007 (1963).
55. C. P. J. Glaudemans, H. G. Fletcher, *J. Org. Chem.*, **28**, 3004 (1963).
56. J. F. Codington, I. L. Doerr, J. J. Fox, *J. Org. Chem.*, **29**, 564 (1964).

Reist and Goodman[57] made ara-G (44, B = guanine) by the fusion of 2,6-dichloropurine and xylofuranose tetraacetate followed by a lengthy series of synthetic steps to arrive at the aranucleoside. They essentially followed the procedure of Lee [52a] and proceeded *via* the 2',3'-epoxide (52). The same group[58] also prepared 5-deoxy-ara-A from its xylo counterpart by tosylation at the 3'-position followed by formation of the lyxoepoxide under basic conditions. Tosylation at the 2'-position led similarly to the riboepoxide. The lyxoepoxide could then be opened by base to yield a mixture of the starting material (5'-deoxyxylo-A) and the desired aranucleoside in a ratio of about 1:2.



Scheme 7.

Moffatt *et al.*[59] took a new approach to the problem (Scheme 7). They protected the 2'- and 5'-hydroxyls of a nucleoside (uridine or cytidine) with trityl groups (54, R = trityl, B = uracil or cytosine). Oxidation using dicyclohexyl-carbodiimide and DMSO led to the 3'-ketouridine (55), which was then reduced with sodium borohydride to give a mixture of the ribo- and xylonucleosides (54 and 45) in a 1:2 ratio. The arabinonucleoside was made in the same way starting from the 3',5'-di-O-trityl ribonucleoside (56). This approach obviates the need to separate α,β-anomeric pairs, and

57. E. J. Reist, L. Goodman, *Biochemistry*, 3, 15 (1964).
58. E. J. Reist, V. J. Bartuska, D. F. Calkins, L. Goodman, *J. Org. Chem.*, 30, 3401 (1965).
59. (a) A. F. Cook, J. G. Moffatt, *J. Am. Chem. Soc.*, 89, 2697 (1967). (b) U Brodbeck, J. G. Moffatt, *J. Org. Chem.*, 35, 3552 (1970).

avoids the tedious synthesis of sugars required for most fusion syntheses. However, the diprotected starting material is difficult to prepare in good yield, since the secondary hydroxyls are almost equally reactive.

Lunzmann and Schramm[60] succeeded in making ara-A (44, B = adenine) as the anomeric mixture by simply heating the free base and arabinose with phenylpolyphosphate in moist acidic DMF. This led to a 3:2 ratio of the anomers, in favour of the α.

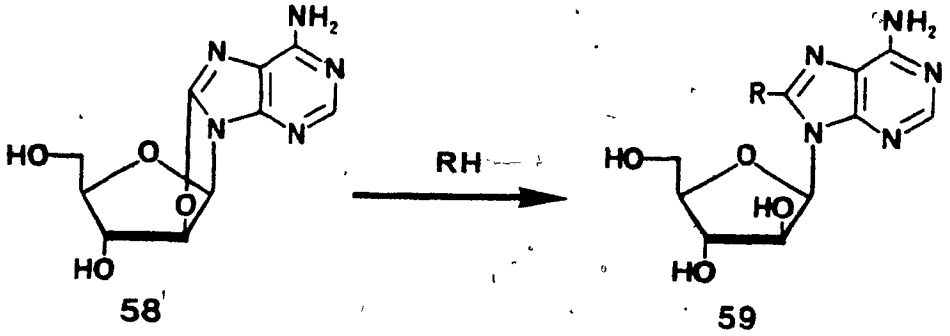
In 1970, Shimizu and Shimizu[61] reported the fusion synthesis of ara-C (44, B = cytosine) by the fusion of 5-O-carboethoxy-3-O-tosyl-2-O-acetylxylofuranosyl chloride with trimethylsilyl-N^4-acetylcytosine in the presence of mercuric bromide.This led to the β anomer of the protected xylonucleoside. Deacetylation led to 2',3'-riboepoxide formation (52, B = cytosine) which was in turn followed by formation of the 2,2'-anhydronucleoside. Alkaline opening of the anhydro link led to the desired aranucleoside. The deacetylation conditions (methanol-water-sodium hydroxide) led directly to the product without isolation of any of the intermediates

Nagyvary and Tapiero[62] made aracytidine-3'-phosphate by mesyl- or tosylchloride-assisted cleavage of the 2',3'-cyclic phosphate (protected at 5' and N^4 by acetyl)to form the 2,2'-anhydronucleoside, which was then opened with aqueous bicarbonate. Alternatively, the 5'-position could be left unprotected, and the base protected with dimethylaminomethylene, and treated in a similar way to give a somewhat higher yield of the arabinonucleotide.

Ogilvie[63] synthesised ara-C (44, B = cytosine) by heating cytidine in moist dimethylformamide with diphenyl carbonate and sodium bicarbonate. 2,2'-Anhydrocytidine is formed, and cleaved *in situ* by hydroxyl ion to yield the desired product.

Xylo-G (45, B = guanine) was synthesised[64] by the fusion of silylated 2-acetamido-6-chloropurine with 2,3,5-tri-O-acetyl-D-xylofuranosyl bromide in the presence of mercuric cyanide followed by treatment with base to remove protecting groups and convert to the 6-oxo form Similarly, fusion with the arabinoside gave the anomeric mixture (1 1) of the arabinonucleoside (44, B = guanine). This mixture was not separated. The same group had previously synthesised[65] xylo G by fusing the protected base with 1,2,3,5-tetra-O-acetylxylofuranoside in the presence of a Friedel-Crafts catalyst (aluminum chloride) and chlorobenzene. This also gave the anomeric mixture In addition, glycosylation of the base occurred at both N^7 and N^9, resulting in a complicated mixture

60. G. Lunzmann, G. Schramm, *Biochim. Biophys. Acta*, **169**, 265 (1968)
61. B Shimizu, F. Shimizu, *Chem. Pharm. Bull*, **18**, 1060 (1970)
62. J. Nagyvary, C. M. Tapiero, *Tetrahedron Lett*, 3481 (1969)
63. K. K. Ogilvie, *Carbo. Res.*, **24**, 210 (1972).
64. W. W. Lee, A. P. Martinez, L. Goodman, D. W Henry, *J Org Chem*, **37**, 2923 (1972).
65. W. W. Lee, A. P Martinez, L. Goodman, *J. Org Chem*, **36**, 842 (1971).



Scheme 8.

Ikehara and Ogiso[66] were able to produce ara-A by the cleavage of 8,2'-anhydro-8-oxy-9-D-arabinofuranosyladenine (**58**) with hydrogen sulfide (Scheme 8). This gave the 8-mercapto derivative of ara-A (**59**, R=SH). Removal of the mercapto group with Raney nickel, or preferably oxidation, gives ara-A (**44**, B=adenine). Chattopadhyaya and Reese[67] used the same 8,2'-anhydroadenosine to make ara-A, this time by hydrazinolysis, followed by treatment with yellow mercuric oxide. Treatment of the 8-hydrazinoara-A (**59**, R=H_2NNH-)with sodium methoxide was equally effective in removing the hydrazino group. The same authors reported[68] that a similar procedure could be used to produce ara-G (**44**, B=guanine) and some of its derivatives.

Robins *et al.*[69] used the riboepoxide route to synthesise xyloadenosine. The 2',3'-riboepoxide (**52**, B=adenine, protected at the 5'-hydroxyl) was opened with sodium benzoate in moist DMF, followed by treatment with sodium methoxide to remove protecting groups, to give the xylonucleoside in good yield. Alternatively, treatment of the 5'-protected xylonucleoside with mesyl chloride, followed by methoxide ion, led to the formation of the lyxoepoxide (**51**, B=adenine), along with deprotection.

A rather long procedure was used by Ranganathan[70] to make ara-A (**44**, B=adenine). An arabinothionoxazolidine was used as the starting material, and the base was built onto the sugar in five steps, in an overall yield of about 40%. The author suggests that, with appropriate substitutions of the starting materials, a variety of purine nucleosides could be constructed *via* this route.

66. M. Ikehara, Y. Ogiso, *Tetrahedron*, **28**, 3695 (1972).
67. J. Chattopadhyaya, C. B. Reese, *J. Chem. Soc. Chem. Comm.*, 414 (1977).
68. J. Chattopadhyaya, C. B. Reese, *Synthesis*, 908 (1978).
69. M. J. Robins, Y. Fouron, R. Mengel, *J. Org. Chem.*, **39**, 1564 (1974).
70. R. Ranganathan, *Tetrahedron Lett.*, 1185 (1975).

A few authors have suggested that the well-known stereospecificity of enzymes could be brought to bear on this problem. A Japanese group[71] demonstrated the synthesis of ara-A (44, B = adenine) by bacterial transglycosylation. In this case, ara-U (44, B = uracil) was used as the arabinose source, and the product was collected by crystallisation after centrifugation to remove the bacteria. The yield, based on added adenine, was better than 90%. Morisawa and coworkers[72] reported a similar method, again using ara-U as the sugar source. In this case, the sugar acceptor was 2-chlorohypoxanthine. The aranucleoside product was then isolated and treated with methanolic ammonia to yield ara-G (44, B = guanine). Another group[73] used 2,2'-anhydrocytidine (27, X = NH, R = H) as the starting material. This was cleaved to yield ara-C (44, B = cytosine) by alkaline aqueous hydrolysis. Without isolation, the cytidine nucleoside was deaminated to ara-U enzymatically, then phosphorolysed to yield the required arabinose phosphate. This was then added, again enzymatically and without isolation, to any of a variety of purine bases, to yield a range of purine arabinonucleosides.

Xylo-U (45, B = uracil) has been converted in moderate yield to xylo-C[74] (45, B = cytosine) by transformation to 4-thioxylo-U, followed by conversion to the cytidine derivative using methanol-ammonia. Deprotection gave the final product in 36% overall yield.

Ogilvie *et al.* reported[75] the synthesis of ara-G (44, B = guanine) by coupling silylated 2-amino-6-chloropurine with 2,3,5-tri-O-benzyl- -D-arabinofuranosyl chloride in the presence of molecular sieves. This gave only the desired -anomer in 33% yield.

At the time we entered this area of work, there was as yet no generally applicable route from natural nucleosides to their arabino- and xylonucleoside counterparts. The fusion route generally gives a mixture of α- and β-anomers. The use of participating groups such as acetyl at the 2-position enhanced the stereoselectivity of the coupling, but was only useful for the synthesis of *trans*-1,2-nucleosides, such as ribo- or xylonucleosides. Transformation to aranucleosides is difficult in some cases. Furthermore, the fusion synthesis requires access to an appropriate glycosyl halide These are often unstable and tedious to prepare.

The enzymatic transformations mentioned above are promising, but require a nucleoside sugar source, and can only handle such bases as are substrates for the enzymes These procedures

71. T. Utagawa, H. Morisawa, T Myoshi, F. Yoshinaga, A. Yamazaki, K. Mitsugi, *Fed. Exp Biol. Soc. Lett.*, 109, 261 (1980).
72. H. Morisawa, T. Utagawa, T Miyoshi, F Yoshinaga, A. Yamazaki, K. Mitsugi, *Tetrahedron Lett.*, 21, 479 (1980).
73. T. A. Krenitsky, G. W Koszalka, J. V Tuttle, J. L. Rideout, G. B Elion, *Carbohydr Res*, 97, 139 (1981).
74. T. Maruyama, S. Sato, M Honjo, *Chem. Pharm. Bull.*, 30, 2688 (1982)
75. U O. Cheriyan, K. K. Ogilvie, *Nucleosides & Nucleotides*, 1, 233 (1982)

also call for microbiological techniques with which most chemists are unfamiliar, and for which they are poorly equipped.

It would seem that the best route to sugar-modified nucleosides is to start with the natural nucleoside. Operating on natural ribonucleosides is somewhat inconvenient, due to the necessity of protecting the various hydroxyl groups.

This was the state of the area in 1981. We felt that a generally applicable synthesis of ara- and xylonucleosides might be useful, particularly if the naturally occurring nucleosides could be used as starting materials. These are relatively inexpensive, and offer most of the desired stereochemistry. However, it is necessary to protect selectively the various hydroxyls. Fortunately, a mild and highly selective protection procedure had recently been developed in our laboratory[76]. The *t*-butyldimethylsilyl protecting group has been very useful, as it may be introduced selectively and in excellent yield at either the 2'- or 3'-hydroxyl. Furthermore, it is stable to a wide variety of reagents, and yet easily and quantitatively removed with fluoride ion under near-neutral conditions. Thus we felt that the way was clear for a general synthesis of the desired nucleosides.

We chose to use the oxidation-reduction approach pioneered by Moffatt (*vide supra*). We felt that, using silyl protecting groups, we had a great deal of leeway in terms of reagents. After preparing the necessary 2',5'- and 3',5'-silylated nucleosides, we embarked on a program of examining a variety of oxidising agents, including the "activated" dimethylsulphoxide systems, and various chromate-based reagents. We then reduced the ketonucleosides to the epimeric mixture of ribo- and ara- or xylonucleosides, and separated them chromatographically. The methods used will be described in the next chapter. Our results were quite good in some cases, disappointing in others. As will be mentioned in the next chapter, we were not the only group exploring this route, and our work was cut short by the publication of very similar results.

Nucleotide Analogues.

We also wished to examine another area of analogue synthesis. As mentioned earlier, chemists have tried to modify every part of the basic nucleotide structure. Sugar modifications have already been discussed above. Another very fruitful area has been the synthesis of nucleotide analogues in which the internucleotide link is altered. Various groups have replaced the phosphodiester link with phosphorothioates, phosphinic acid derivatives, carbonates, carbamates, amides, and a variety of other functions. Some of these will now be described.

76. G. H. Hakimelahi, Z. A. Proba, K. K. Ogilvie, *Can. J. Chem.*, **60**, 1106 (1982).

Nucleotide Analogues.

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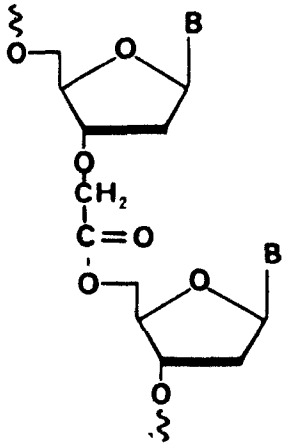

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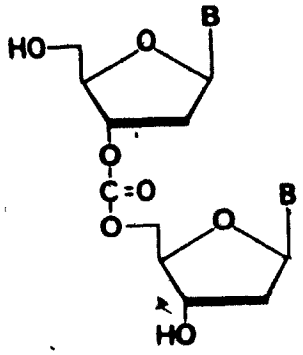


60

The research group of Walker and Jones, with a variety of coworkers has made extensive studies of nucleotides containing carboxymethyl links (**59**). A dimer was produced *via* the DCC-assisted coupling of 5'-O-carboxymethyl-2',3'-O-isopropylideneuridine with 5'-O-tritylthymidine[77] 5'-tritylthymidine was the starting material for the synthesis of 3'-O-carboxymethylthymidine, which could be polymerised by DCC to yield a mixture of homopolymers of various lengths. It was noted that these polymers showed an interaction with polyadenylic acid in water and in salt solutions.

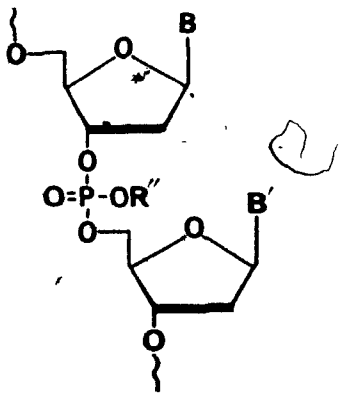
Other oligomers have been made by this group, including the carboxymethyl-linked analogue of various trimers[78] containing two thymidines followed by adenosine, guanosine,

77. (a) M. H. Halford, A.S. Jones, *J. Chem. Soc. C*, 2667 (1968) (b) ----------, *Nature*, 217, 638 (1968).

78. M. D. Edge, A. S. Jones, *J. Chem. Soc. C*, 1933 (1971).

uridine, inosine, or cytidine. Jones has also synthesised analogues[79] in which 3'-carboxymethyladenosine is polymerised with DCC. The extent of polymerisation is controlled by addition of 2',3'-O-protected adenosine. Other polymers were reported in the same publication, made by copolymerising acrylamide with 5'-O-acryloyluridine, and by the polymerisation of polyacrylic acid hydrazide with adenosine dialdehyde. This report also described the inhibition of binding of tRNA to ribosomes by these analogues. The authors speculated that such analogues may interfere with protein synthesis. The same group has published a series of reports on the synthesis of various carboxymethyl-containing analogues and their interactions with various natural nucleotides[80].

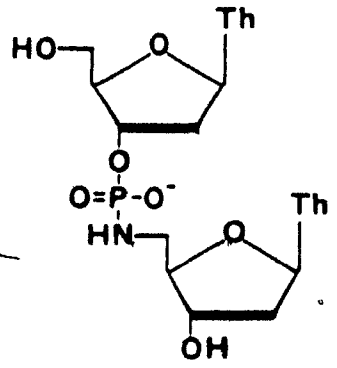
There have also been reports of carbonate-linked nucleotide analogues (**60**). Mertes and Coates[81] synthesised the carbonate analogue of a thymidine dinucleotide, as well as the dinucleotide analogues 3'-thymidinyl-5'-(5-fluoro-2'-deoxyuridinyl)carbonate, and 3'-(5-fluoro-2'deoxyuridinyl)-5'-thymidinyl carbonate. This was done by reaction of a 5'-tritylated nucleoside with phosgene to yield the 3'-chloroformate, followed by reaction with the second (unprotected) nucleoside. Jones and Tittensor[82] reported a dinucleotide carbonate synthesis using a 5'-O-protected-3'-carbonate active ester (the trichloroethyl ester) which was coupled with a 2',3'-protected nucleoside.



5

79. G. J. Cowling, A. S. Jones, R. T. Walker, *Biochim. Biophys. Acta*, **254**, 452 (1971).
80. (a) M. D. Edge, A. Hodgson, A. S. Jones, R. T. Walker, *J. Chem. Soc. Perkin Trans. I*, 1991 (1972). (b) A. S. Jones, M. MacCoss, R. T. Walker, *Biochim. Biophys. Acta*, **294**, 365 (1973). (c) M. J. Gait, A. S. Jones, R. T. Walker, *J. Chem. Soc. Perkin Trans. I*, 1684 (1974). (d) R. C. Bleaney, A. S. Jones, R. T. Walker, *Nucl. Acids Res.*, **2**, 699 (1975).
81. M. P. Mertes, E. A. Coates, *J. Med. Chem.*, **12**, 154 (1969).
82. D. S. Jones, J. R. Tittensor, *J. Chem. Soc. Chem. Comm.*, 1240 (1969).

The above analogues are all nonionic. The natural nucleotide, being a diester of phosphoric acid, is ionised at physiological pH. Many other nonionic nucleotide analogues have been reported. Among these are the nucleotide phosphotriesters (5), mentioned above as intermediates isolated during most of the more recent forms of nucleotide synthesis. Usually these are deprotected to the diester stage. In some cases, however, the triester is the desired product. For example, Miller and coworkers[83] isolated the methyl and ethyl phosphotriesters (5, R" = Me or Et) of thymidine and deoxyadenosine dinucleotides. They reported that proton NMR, circular dichroism and ultraviolet spectroscopic studies showed these molecules to be conformationally similar to their diester counterparts, with the Rp diastereoisomer showing a greater similarity than the Sp. It is important to note that these molecules have a chiral center at phosphorus, and the diastereoisomers formed are often separable. These authors reported that the duplex formed between the triester and diester sequences was often more thermally stable than that formed between two diester sequences, and attributed this to lack of repulsion between negatively charged phosphates.



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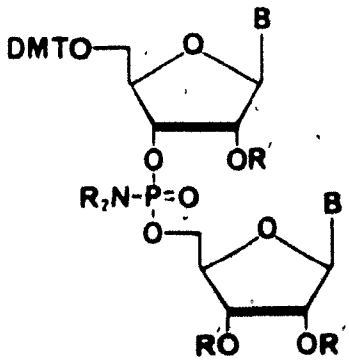


62

Another group has reported[84] that the removal of methyl triesters from alkylated DNA by repair enzymes from *E. coli* is dependant upon the stereochemistry of the triester link. They found that only phosphotriesters with the S configuration were removed.

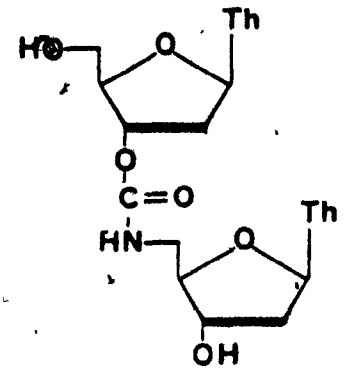
Various groups have reported different syntheses of phosphotriester-linked nucleotides Ste: *et al.*[85] isolated various nucleotide triesters ranging in length from two to five units, and bearing

83. P. S. Miller, K. N. Fang, N. S. Kondo, P O P Ts'o, *J. Am Chem. Soc.*, 93, 6657 (1971)
84. M. Weinfeld, A. F. Drake, J K. Saunders, M. C. Paterson, *Nucl. Acids Res.*, 13, 7067 (1985)
85. W. J. Stec, G. Zon, K. A. Gallo, R A. Byrd, *Tetrahedron Lett.*, 26, 2191 (1985)

isopropyl phosphotriesters (5, R = *i*-Pr) by HPLC. This group was able to establish the absolute configurations of many of the chiral phosphates by the conversion of thiono triesters into both the alkyl triester of interest, and the phosphorothioate, by stereoselective means. Routes have been developed by others (see the section below on the phosphorothioates) for the assignment of the absolute configurations of the thioates. This group has also carried out other studies wherein they examined the effect of stereochemically defined phosphotriester links inserted in various nucleotide sequences upon the stability of duplexes[86]. They also have reported on a simple solid-phase synthesis of these molecules[87].

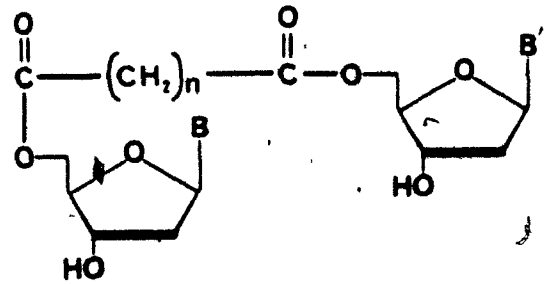
Various other modifications to the internucleotide link have also been made. Letsinger and Mungall[88] made phosphoramidate-linked nucleotides (**61**) by the coupling of of 5'-amino-5'-deoxythymidine with 5'-protected thymidine *via* the phosphate triester procedure. Dimers and trimers were made by this route and found to be stable to mild alkaline hydrolysis, but cleaved by acid and by various phosphodiesterases. In a study on the covalent linking of proteins and nucleic acids, Juodka and Stec[89] made diribonucleotides which bore various amino acids at the phosphorus (**62**). They were, however, unable to deprotect them without cleaving this phosphoramidate link. The problem seemed to involve attack by the 2'-hydroxyl. Juodka has published a review concerning the subject of covalent interactions between proteins and nucleic acids[90].

86. (a) K. A. Gallo, K. Shao, L. R. Phillips, J. B. Regan, M. Koziolkiewicz, B. Uznanski, W. J. Stec, G. Zon, *Nucl. Acids Res.*, 14, 7405 (1986). (b) M. F. Summers, C. Powell, W. Egan, R. A. Byrd, W. D. Wilson, G. Zon, *Nucl. Acids Res.*, 14, 7421 (1986).
87. B. Uznanski, M. Koziolkiewicz, W. J. Stec, G. Zon, K. Shinozuka, L. G. Marzili, *Chem. Scripta*, 26, 221 (1986).
88. R. L. Letsinger, W. S. Mungall, *J. Org. Chem.*, 35, 3800 (1970).
89. B. A. Juodka, J. Smrt, *Coll. Czech. Chem. Comm.*, 39, 963 (1974).
90. B. A. Juodka, *Nucleosides & Nucleotides*, 3, 445, (1984).



63a

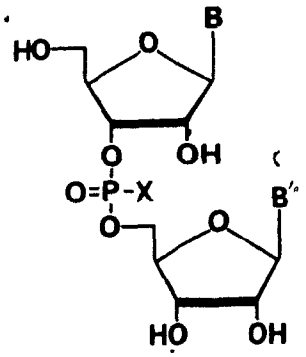
Letsinger[91] returned to the synthesis of phosphoramidate-linked nucleotides in 1975 to make trimers of thymidine by coupling the 5'-O-protected-3'-O-diethylphosphite of thymidine with 5'-deoxy-5'-azidothymidine. Mungall and Kaiser[92] made carbamate-linked dimers **63a** and trimers of thymidine by reaction of 5'-deoxy-5'-aminothymidine with thymidine bearing an activated carbonate ester at the 3'-position. These molecules were shown to be stable to acid and base, as well as snake venom and spleen phosphodiesterases.



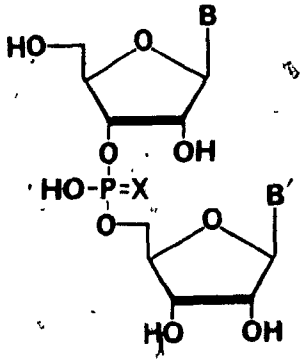
63

91. R. L. Letsinger, G. A. Heavner, *Tetrahedron Lett.*, 147 (1975).
92. W. S. Mungall, J. K. Kaiser, *J. Org. Chem.*, 42, 703 (1977).

As part of a study on inhibition of phosphokinases, enzymes which carry out phosphorylation reactions, Lin and coworkers[93] synthesised possible transition state analogues (**63**). Dimers of either deoxyuridine, thymidine or thymidine and deoxyadenosine, joined in a 5'-to-5' fashion were produced by reaction of the 5'-deoxy-5'aminonucleosides with dicarboxylic acids of various lengths. The coupling was carried out using the diacid chloride. Alternatively, the methyl ester of the acid chloride could be used. This allows selective reaction to introduce two different nucleosides. The methyl ester is cleaved with base and the acid converted to the p-nitrophenyl ester. This is followed by coupling with the aminonucleoside. These authors did not report whether or not any enzyme inhibition was observed.



64



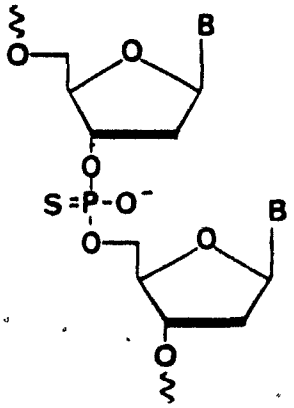
65

Ogilvie and Nemer made a number of modifications based on the reaction of dinucleoside phosphites with various electrophiles. Thus a fully protected diuridine trichloroethylphosphite (**5**, B=B'=uracil, R=trichloroethyl) was converted to the H-phosphonate (**64**, X=H) by removal of the trichloroethyl group using Zn/Cu[94]. These same authors transformed the same starting material into the phosphoramidate (**64**, $X=NR_2$)derivative by reaction with various alkylamines and iodine. Oxidation of the trichloroethyl-protected dinucleoside phosphite (**9**, B=B'=uracil, R=trichloroethyl) with elemental sulphur or selenium in DMF led to the corresponding phosphorothioate (**65**, X=S) or selenate (**65**, X=Se). The hydrogen phosphonate previously

93. T.-S. Lin, G. T. Shiau, W. H. Prusoff, J. P. Neenan, *J. Carbohydr. Nucleosides Nucleotides*, 7, 389 (1980).
94. K. K. Ogilvie, M. J. Nemer, *Tetrahedron Lett.*, 21, 4145 (1980).

described could be converted to the phosphoramidate by reaction with ammonia. Dinucleoside methylphosphonates (of which more will be said later) were made by the Arbuzov reaction of the methyl-protected dinucleoside phosphite with methyl iodide[95]. In the third of this series of papers, thio, seleno, and imino dinucleoside phosphoramidites are described[96]. These are made by reaction of the N, N-diethyl dinucleoside phosphoramidite with sulphur, selenium, or iodine-alkylamine, respectively.

The synthesis and properties of oligonucleotide phosphorothioates (66) have been extensively studied in recent years. These nucleotide analogues have been used to explore the stereochemistry of phosphodiester-cleaving enzymes. The area was reviewed by Eckstein[97] fairly recently. This review outlines the synthesis of analogues of nucleotide mono-, di- and triphosphates as well as oligonucleotide analogues.



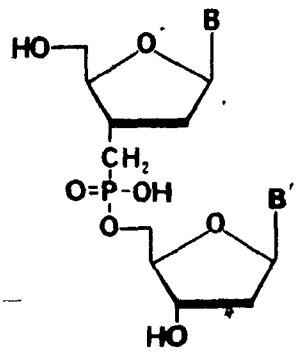
66

Two routes to the synthesis of di- (and thence oligo-) nucleoside phosphorothioate analogues are described in Eckstein's review. In the first, a type of phosphate triester chemistry, involves the reaction of β-cyanoethyl phosphorothioate with the 3'-hydroxyl of the first nucleoside (protected at the 5'-position) in the presence of an appropriate condensing agent[98]. This product is condensed, again in the presence of a coupling agent, with the 5'-hydroxyl of the second nucleoside. In these molecules, the phosphorus is chiral, and in this particular example, the diastereoisomers could be separated after 5'-deprotection. The second route is analogous to phosphite synthesis of

95. K. K. Ogilvie, M. J. Nemer, *ibid.*, 21, 4149 (1980).
96. K. K. Ogilvie, M. J. Nemer, *ibid.*, 21, 4153 (1980).
97. F. Eckstein, *Angew. Chem. Int. Ed. Eng.*, 22, 423 (1983).
98. P. M. J. Burgers, F. Eckstein, *Biochemistry*, 18, 592 (1978).

phosphite synthesis of natural nucleosides. The phosphorylating agent in this case is methylphosphodichloridite. The reaction proceeds as described above for phosphite synthesis, and sulphur is introduced by oxidation of the phosphite triester intermediate with elemental sulphur[99]. Frey[100] has reviewed the methods of determination of the stereochemistry of phosphorothioates and their use in the determination of enzyme reaction stereochemistry.

Phosphonic acid analogues of natural products have been reviewed by Blackburn[101] and this class of analogues as it applies to the replacement of phosphodiesters has been reviewed by Engel[102].



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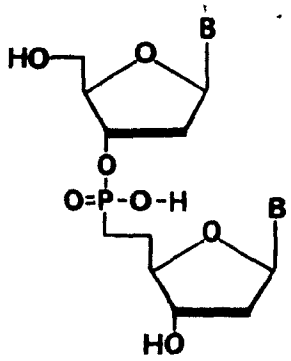
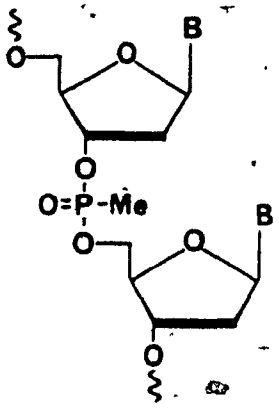


67

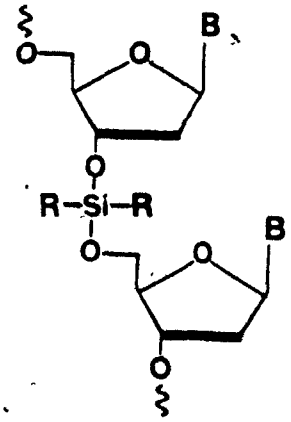
Most of the earlier work in the area of phosphonic acid analogues of nucleotides was on the synthesis of mono-, di- and triphosphate analogues. The first report of a dinucleotide phosphonate was that of Jones *et al.*[103]. These workers made dimers of adenosine and uridine of two types. In one type (66), the 3'-hydroxyl involved in the internucleotide link was replaced by methylene. In the second type (67), the 5'-hydroxyl of the link was replaced by methylene. In both cases the synthesis was accomplished by a modification of traditional diester chemistry. The nucleoside 3'- or 5'-phosphonate was coupled with the appropriately protected nucleoside to yield the 3',5'-linked dimer. DCC was used as the coupling agent. The initial phosphonate was

99. J. F. Marlier, S. J. Benkovic, *Tetrahedron Lett.*, 21, 1121 (1980).
100. P. A. Frey, *Tetrahedron*, 38, 1541 (1982).
101. G. M. Blackburn, *Chem. Ind. (London)*, 134 (1981).
102. R. Engel, *Chem. Rev.*, 77, 349 (1977).
103. G. H. Jones, H. P. Albrecht, N. P. Damodaran, J. G. Moffatt, *J. Am. Chem. Soc.*, 92, 5510 (1970).

DCC was used as the coupling agent. The initial phosphonate was synthesised by coupling the base to the furanosyl halide which had previously been phosphonylated by a Wittig reaction.



68



69

A considerable amount of work has been done on the preparation of di- and oligonucleotide phosphonate analogues in which phosphorus bears an alkyl or aryl group in place of one of the "non-nucleoside" oxygens (68). These analogues are neutral, and contain a chiral center at phosphorus. Ts'o *et al.* published the first report of a dinucleoside methylphosphonate[104]. The authors described the synthesis of the four possible 3',5'-linked dimers of deoxyadenosine and thymidine (68, B = thymine or adenine). The route used involved the condensation of methylphosphonic acid with the 3'-hydroxyl of the 5'-protected nucleoside. The nucleoside phosphonate or its -cyanoethyl ester was isolated and coupled with the 5'-hydroxyl of the second nucleoside, protected at the 3'-position with acetyl. Use of the -cyanoethyl ester allowed for easier purification of the intermediate. This group carried out CD and UV studies on these dimers, and on their interaction with complementary natural nucleotides. In this and a later paper[105], extensive proton NMR studies were undertaken to determine the solution conformation of these molecules.

Other routes to these analogues have been reported. Agarwal and Riftina[106] made both methyl- and phenylphosphonate deoxynucleotides by several different (modified phosphate triester) routes. They found that the use of methylphosphonodichloridate as phosphonylating agent led to

104. P. S. Miller, J. Yano, E. Yano, C. Carroll, K. Jayaraman, P. O. P. Ts'o, *Biochem.*, 18, 5134 (1979).
105. L S. Kan, D. M. Cheng, P. S. Miller, J. Yano, P. O. P. Ts'o, *Biochem.*, 19, 2122 (1980).
106. K. L. Agarwal, F. Riftina, *Nucl. Acids Res.*, 6, 3009 (1979).

low yields, but that the reaction of methylphosphonoditetrazolide with the 3'-hydroxyl of the first nucleoside, followed by addition of the second (3'-protected) nucleoside in the presence of benzenesulfonyl tetrazole, led to good yields of the desired 3', 5'-linked dimers. The phenylphosphonates were made by the same route. Another group has taken a similar approach, using methyl-O,O-bis-(1-benzotriazolyl)phosphate as the phosphonylating reagent[107].

Miller *et al.*[108] synthesised oligonucleotides in which the links alternated between phosphodiester and methylphosphonate. The phosphonates in a given sequence had the same stereochemistry. This was achieved by preparing dimers and separating the diastereoisomers chromatographically, then coupling them in block fashion *via* diester links.

Engels and Jaeger[109] reported a solution synthesis of the methylphosphonate analogue of the thymidine dimer (68, B = thymine, R = Me) using methyldichlorophosphine, which is reacted with the 3'-hydroxyl of 5'-O-tritylthymidine, then with 3'-O-benzoylthymidine. The resulting dimer is oxidised *in situ* with *t*-butyl peroxide to give the diasteromeric mixture in good yield. This route was adapted to solid-phase synthesis by Koster *et al.*[110] (using iodine-water oxidation) who made sequences up to four units long, and studied their use as enzyme substrates, as well as their stability to alkali. Haeger and Engels later reported [111] the use of deoxynucleoside 3'-methylphosphonamidites as intermediates in the synthesis of this class of analogues. These compounds are stable and easily stored for long periods. Like nucleoside phosphoramidites, they are coupled with the 5'-hydroxyl of an appropriately protected nucleoside in the presence of a weak acid. Again, the yields are quite good.

Some attempts at synthesis of nucleoside phosphonates *via* Arbuzov reactions have been reported. Ogilvie and Nemer reacted methyl iodide with a dinucleoside phosphite, as mentioned above, to make the methylphosphonate in good yield. However, Caruthers *et al.* found that this reaction proceeded in only very low yield when applied to polymer bound sequences[112]. Another group found similar results, but also found that dimethoxytritylphosphonates (68, R = dimethoxytrityl) could be produced by the Arbuzov reaction of dimethoxytrityl chloride with

107. J. Marugg, E. de Vroom, C. E. Dreef, M. Tromp, G. A. van der Marel, J. H. van Boom, *Nucl. Acids Res.*, **14**, 2171 (1986).
108. P. S. Miller, N. Dreon, S. M. Pulford, K. B. McParland, *J. Biol. Chem.*, **255**, 9659 (1980).
109. J. Engels, A. Jaeger, *Angew. Chem. Int. Ed. Eng.*, **21**, 912 (1982).
110. N. D. Sinha, V Grobbruchhaus, H. Koster, *Tetrahedron Lett.*, **24**, 877 (1983).
111. A. Haeger, J. Engels, *Tetrahedron Lett.*, **25**, 1437 (1984).
112. S. A.Noble, E. F. Fisher, M. H. Caruthers, *Nucl. Acids Res.*, **12**, 3387 (1984).

various oligonucleoside phosphites, in fairly low yield. This was the serendipitous result of studies relating to other Arbuzov reactions[113].

Ts'o *et al.* have reported two solid phase syntheses of oligonucleoside phosphonates. In the first[114], methyl phosphonic dichloride was allowed to react with the 3'-hydroxyl of the 5'-protected nucleoside. The resulting nucleoside 3'-methylphosphonic chloride was purified on silica gel. It was then added to the free 5'-hydroxyl of the growing, silica support-bound sequence. Yields were fairly good when oligothymidine sequences were being made, but dropped precipitously for other nucleosides. The authors turned to the use of the triethyl ammonium salt of the nucleoside 3'-methyl phosphonate as the reactive intermediate, added to the growing nucleotide phosphonate chain[115]. In this case, good yields were obtained with all nucleosides tried (average coupling yields in the range of 74-86%). Members of this group have obtained a patent on the synthesis and use of oligonucleoside aryl- and alkylphosphonates[116].

As mentioned earlier, these various phosphate modified nucleotide analogues are made for a number of reasons. Frequently, they are designed to explore the mechanisms and stereochemistry of enzymatic reactions. This is especially true of the phosphorothioates, as outlined in the reviews by Eckstein[97] and Frey[100]. They have been used to show the stereochemistry of the hydrolysis reactions of over fifty phosphodiesterases. They have also been used as references for determining the stereochemistry of other analogues[85], and as starting points for the stereospecific synthesis of isotopically labelled phosphates[100].

Many oligonucleotide analogues are synthesised in order to study the factors important in the binding of nucleic acid strands. The formation of these duplexes is of primary importance in the function of nucleic acids. It is what stabilises the DNA double helix, and allows the transcription of DNA into RNA as outlined at the beginning of this introduction. Some groups have studied the effect of nucleotide analogue binding on this transcription. Ts'o *et al.*[117] have shown that addition of nucleotide oligomethylphosphonate analogues to both cultured cells, and cell-free translation

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113. W. J. Stec, G. Zon, W. Egan, R. A. Byrd, L. R. Phillips, K. A. Gallo, *J. Org. Chem.*, 50, 3908 (1985).
114. P. S. Miller, C. H. Agris, M. Blandin, A. Murakami, P. M. Reddy, S. A. Spitz, P. O. P. Ts'o, *Nucl. Acids Res.*, 11, 5189 (1983).
115. P. S. Miller, C. H. Agris, A. Murakami, P. M. Reddy, S. A. Spitz, P. O. P. Ts'o, *ibid.*, 11, 6225 (1983).
116. P. O. P. Ts'o, P. S. Miller, U. S. Pat. 4,469,863 (1984).
117. (a) K. R. Blake, A. Murakami, S. A. Spitz, S. A. Glave, M. P. Reddy, P. O. P. Ts'o, *Biochemistry*, 24, 6139 (1985). (b) P. S. Miller, C. H. Agris, L. Aurelian, K. R. Blake, S.-B. Lin, A. Murakami, M. P. Reddy, C. Smith, P. O. P. Ts'o, *Interrelationship Among Aging, Cancer and Differentiation*, B. Pullman, ed., D. Reidel Pub. Co., 207-219 (1985). (c) P. S. Miller, C. H. Agris, L. Aurelian, K. R. Blake, A. Murakami, M. P. Reddy, S. A. Spitz, P. O. P. Ts'o, *Biochimie*, 67, 769 (1985).

systems, can severely inhibit the translation of the mRNA sequences to which the analogues are complementary. For example, this group has shown that phosphonate analogues complementary to initiator regions of various rabbit globin protein mRNAs can inhibit the synthesis of the corresponding globins both in reticulocytes and in cell-free lysates.

Furthermore, it has been pointed out that the therapeutic activity of many nucleoside analogues depends upon their being converted in the target cell to the nucleotide form. Some infected or malignant cells develop resistance to these drugs by losing the ability to carry out this transformation. Thus it has been suggested that administration of the drug in the nucleotide form would be a way of circumventing such resistance. Natural nucleotides are, however, ionised at physiological pH (7.4) and thus do not cross the cell membrane readily. Hence the logic of synthesising neutral analogues. Bennett[118] has recently pointed out that this may not be such a good route after all, since the healthy cells in the organism will probably take up the prodrug in at least comparable concentrations to those in the target cell. Thus, it may be necessary to use prodrugs which are more specific to the target cell, or virus. In the long run, analogues corresponding to some critical part of the genetic sequence of the virus or tumor cell may be useful. This, however, is a far-off goal.

In the meantime, analogues such as the oligonucleotide alkylphosphonates may give important clues as to the control of genetic expression, by their ability, described above, to turn various sequences "on" or "off". It will be important to find analogues which bind very tightly, even irreversibly, in order to exert effective control over gene expression. As part of this thesis, work toward a nucleotide analogue (69) in which the phosphodiester link is replaced by a dialkyl- or diarlysilyl ether bridge, will be described. In addition to synthetic work, and characterisation, some speculation will be made as to how to handle the problem in future. This work will be described in detail later in this thesis.

Briefly, a variety of alkyl- and arylsubstituted silylating agents were used to form 3',3'-, 5',5'- and 3',5'-linked silyl nucleotide analogues (69, B = adenine or thymine, R = alkyl or aryl). It was necessary to examine the problem of protecting groups carefully, since the silyl links are somewhat sensitive to both acidic and basic conditions. After preparing a variety of the dimers, we determined that the diisopropylsilyl link was sufficiently stable to carry out extended synthesis. Oligomers of thymidine and deoxyadenosine up to six units long were synthesised and studied by UV and CD. These results indicated that these molecules were capable of forming helices. Solubility problems made it impossible to examine the ability of the analogues to form duplexes with natural nucleotides.

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118. L. L. Bennett, R. W. Brockman, J. A. Montgomery, *Nucleosides & Nucleotides*, 5, 117 (1986).

Chapter 1.

Synthesis of ara- and xylonucleosides.

Introduction.

As described in the preceding general introduction, there have been many approaches to the synthesis of sugar modified nucleosides, and thence to modified nucleotides. Various modified nucleic acid components have been used as chemotherapeutic agents and as probes for studying the biological functions of nucleic acids. The use of ara-C as an antileukemic and ara-A in antiviral chemotherapy are well known. Some of the altered nucleosides used as antiviral agents were also described in the introduction. Many different, and some quite ingenious, routes to these molecules have been described in the chemical literature. There has not been, however, a generalised route to these analogues, and, in particular, to the ara- and xylonucleosides. The biological activity of this particular class of nucleosides and their derivatives has stimulated a great deal of synthetic chemistry. The work done in the past has, however, been somewhat scattered and haphazard, relying mainly on the properties of the individual nucleosides. We felt that a more comprehensive approach would be useful, especially in view of the use of some of these compounds clinically.

We hoped to develop a route which took advantage of readily available starting materials, was straightforward, and involved a minimal number of synthetic steps. In addition, the route should should be broadly applicable, irrespective of the base involved. With this in mind, we considered both the fusion and nonfusion routes mentioned in the introduction (pp. 23-24).

In the fusion route, it is necessary to prepare an appropriately protected sugar bearing the correct stereochemistry, and fuse it with the desired base. The preparation of the sugar is often tedious, and the fusion may result in an anomeric mixture which is difficult to purify In sugar chemistry, the introduction of a substituent at the 1-position (in this case the heterocyclic base) in a *cis* relationship with that at the 2-position (to give an arabinonucleoside), often involves several steps after the coupling reaction. This is due to the fact that, in many cases, it is necessary to use participating protecting groups in order to obtain the desired anomeric configuration (Baker's *trans* rule[47], Scheme 4). As outlined in the general introduction to this thesis, this normally gives the 1- and 2-substituents the *trans* orientation with respect to one another. Various inversions are then required to arrive at the desired 1,2-*cis* orientation (see, for example, the syntheses of ara-A by Baker *et al.* referred to in the introduction[52]. Contamination with the xylonucleoside may also occur. The choice of protecting groups may be limited by the need to have the protecting group

participate in the fusion reaction. Finally, the coupling reaction itself often involves the use of mercury or silver salts, which are difficult to completely remove if biological studies are contemplated. Thus, we hoped to avoid this route.

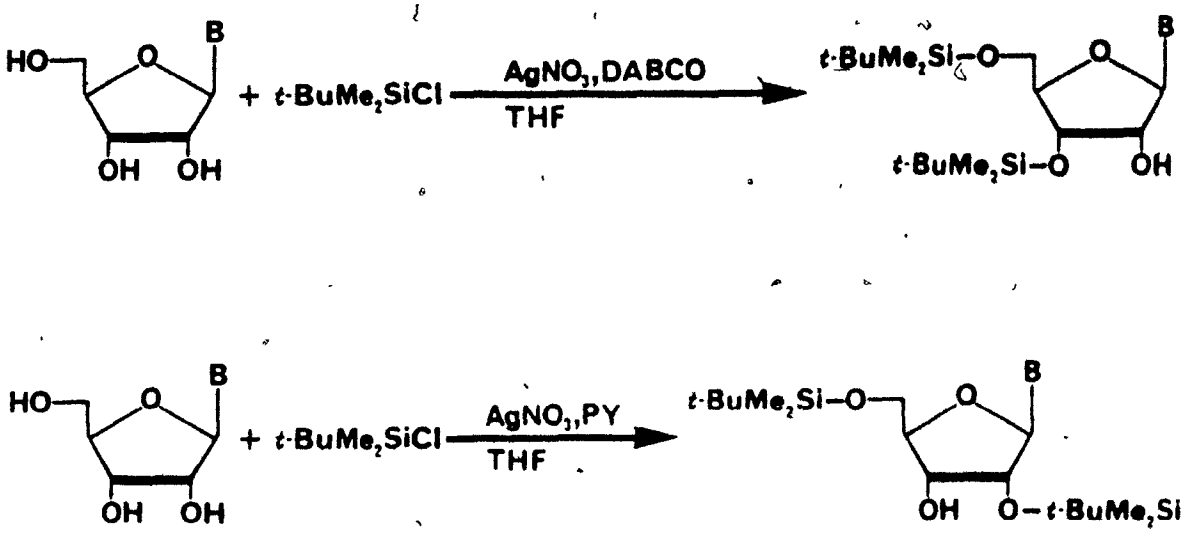
The alternative method, operating on the preformed nucleoside, is also fraught with difficulties. Anhydronucleoside formation may interfere with reactions in which displacement of (*e.g.*) a tosylate or mesylate is desired. Of course, this reaction may be turned into an advantage, as described in the introduction. For example, Ogilvie synthesised ara-C by opening of the corresponding anhydronucleoside[63], but this is only possible with some nucleosides, mainly pyrimidines, which can readily form the required anhydronucleoside, as described in the general introduction. Selective protection of the secondary hydroxyls of ribonucleosides may be a problem, since their reactivity is very similar. Attempts at selective protection usually lead to mixtures which may be difficult to separate, and which limit the efficiency of any synthesis.

Synthetic approach.

At the time we began this project, a highly selective silylation procedure had just been developed in our laboratory[76]. This allowed the introduction, in good yield, of the very useful *t*-butyldimethylsilyl protecting group at either the 2'- or 3'-position (Scheme 9). We felt that we could take advantage of this facility. The silyl protecting group is stable to a wide range of conditions, and removed quantitatively and rapidly with fluoride ion under near-neutral conditions. It is also amenable to the conditions used for nucleotide synthesis, should we wish to use our products for this purpose. The disilylated nucleosides are easily purified, with the exception of the case of guanosine.

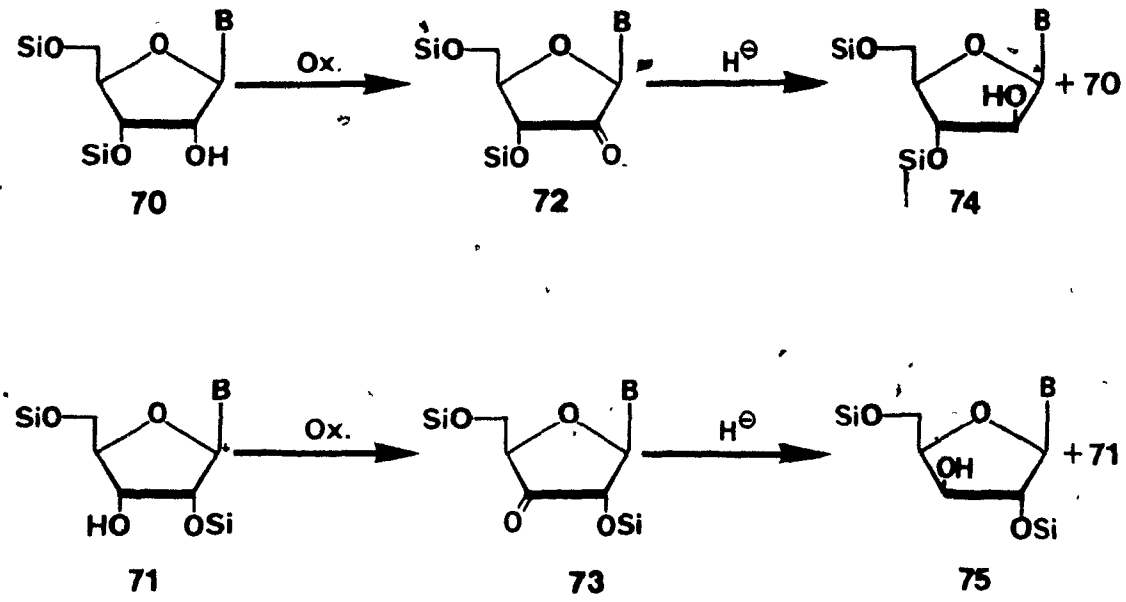
The 2',5'- and 3',5'-di-O-*t*-butyldimethylsilyl isomers of guanosine are quite difficult to separate, except when triethylamine is used in the chromatographic solvent, and these basic conditions may promote isomerisation of the silyl group at the secondary hydroxyl. It had previously been found in this laboratory that the corresponding triisopropylsilyl-protected guanosines were much more easily purified. Hence, we used the triisopropylsilyl group for guanosine hydroxyl protection[118].

118. (a) K. K. Ogilvie, K. L. Sadana, E. A. Thompson, M. A. Quillam, J. B. Westmore, *Tetrahedron Lett.*, 2861, (1974). (b) Ph. D. Thesis, S. Boisvert, McGill University, 1987.



Scheme 9.

We decided that it would be useful to take up the general method first pioneered by the research group of Moffatt[59]. These workers oxidised the ditritylated nucleoside to the ketonucleoside (54 and 56, R = trityl, B = uracil or cytosine), then reduced to give a mixture of starting material and ara- or xylonucleosides (57 and 55, R = trityl, B = uracil or cytosine), depending upon the protection scheme, with the emphasis on the latter configurations. However, the preparation of the required ditritylated nucleoside precursors 54 and 56 is unselective, leading to low overall yields. In view of our ability to prepare the required protected nucleosides selectively, however, this general methodology looked quite promising. We decided to try to prepare both the 2'- and 3'-keto derivatives (72 and 75, a-f)of all four common ribonucleosides. We would then reduce the carbonyl, and separate the resulting mixture of ribo- and ara- or xylonucleosides. We felt that this approach (Scheme 10), if successful, would be amenable to use with a variety of different nucleosides. Since the process does not depend on the opening of an anhydronucleoside such as 24 or 25, it would be useful for both purines and pyrimidines. The protected nucleosides are easily accessible, and we hoped to use inexpensive reagents which required a minimum of precautions with regard to moisture and whose use did not require particularly vigourous reaction conditions.



B, Si = a, uracil, *t*-$BuMe_2Si$-; b, N^4-benzoylcytosine, *t*-$BuMe_2Si$-; c, N^6-benzoyladenine, t; d, N^2-benzoylguanine, *i*-Pr_3Si-; e, adenine, *t*-$BuMe_2Si$-; f, cytosine, *t*-$BuMe_2Si$-.

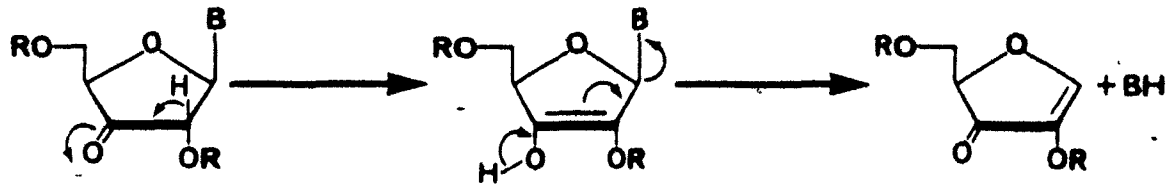
Scheme 10.

Ketonucleosides have been reported several times in the chemical literature. For example, in addition to the Moffatt papers mentioned above, another group reported the synthesis of the 3'-keto derivative of thymidine by a photochemical oxidation[119]. 3',5'-Protected xyloadenosine was oxidised to the 2'-ketone with ruthenium tetroxide as the first step of a synthesis of aminosugar nucleosides[120]. The ketonucleoside was isolated but not deprotected. 2'- and 3'-ketoadenosines were produced by the DMSO-DCC oxidation of the monomethoxytrityl-protected nucleosides[121]. Careful acid treatment allowed the removal of the protecting groups and isolation of the free ketonucleosides. These authors reported that these products were somewhat unstable in aqueous solution, with $t_{1/2}$ of 11 and 84 hours for the depurination of the 2'- and 3'-ketoadenosines, respectively.

119. R. W. Binkley, D. G. Hehemann, W. W. Binkley, *J. Org. Chem.*, 43, 2573, (1978).
120. A. Rosenthal, M. Sprinzl, D. A. Baker, *Tetrahedron Lett.*, 4233, (1970).
121. R. P. Crews, D. C. Baker, *Nucleosides & Nucleotides*, 2, 275, (1983).

Synthesis of protected ketonucleosides.

The first problem we faced was to find an acceptable oxidising agent. There are a great many agents in the literature which can be used to transform a secondary hydroxyl into a carbonyl. We wished to use a relatively mild reagent which would still give quantitative or near-quantitative yields, minimising the handling, and especially chromatographic purification, of the intermediate ketonucleoside. These molecules are somewhat unstable, especially with the carbonyl in the 3'-position, where it is susceptible to enolisation and loss of the heterocyclic base by -elimination (Scheme 11). This was first observed by Moffatt[59]. Clearly, extremes of basicity are to be avoided here. Furthermore, nucleosides in general, and adenosine derivatives in particular, are susceptible to acid-promoted depurination[122], so that care should be taken to avoid even mildly acidic conditions if long reaction times are needed.

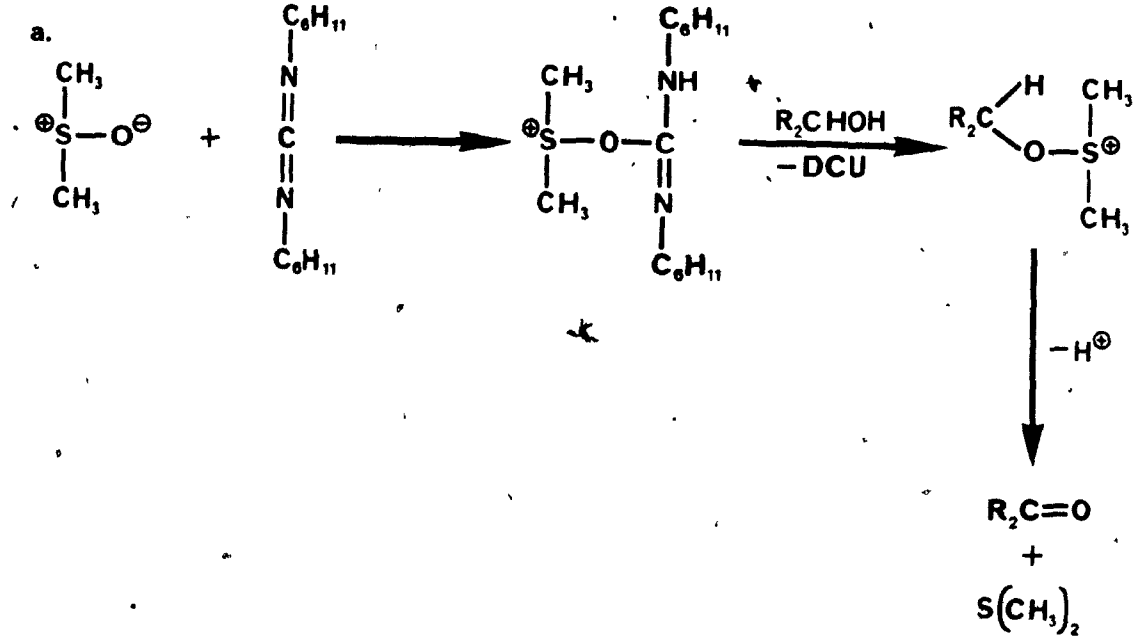


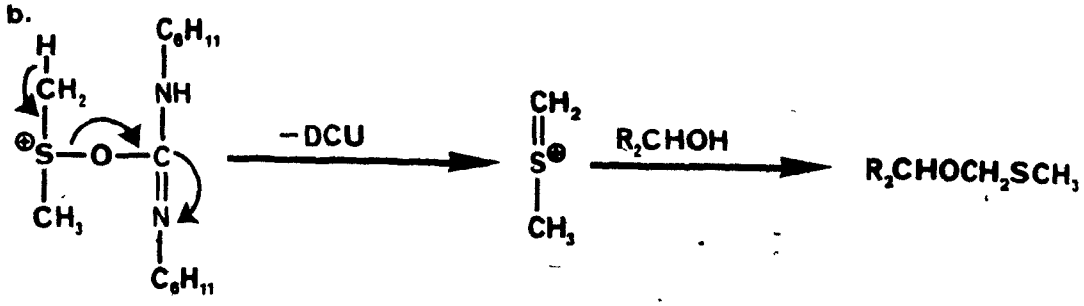
Scheme 11.

There has been a great variety of "activated DMSO" oxidising reagents reported in the literature which are characteristically mild. We initially examined the Pfitzner-Moffatt DMSO-DCC procedure[123] (Scheme 12a). We tried this reaction with 2',5'- and 3',5'-disilylated uridine (**71a** and **70a**) as substrates.

122. (a) P. T. Gilham, H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212 (1958) (b) H. Schaller, H. G. Khorana, *ibid.*, **85**, 3828 (1963).

123. (a)K. E. Pfitzner, J. G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5661 (1965). (b) ----------, *ibid.*, **85**, 5670, (1965).


a.
C_6H_{11}
CH_3
$\oplus S - O^{\ominus}$
CH_3
+
N
C
N
C_6H_{11}
NH
O
R_2CHOH
$-DCU$
H
R_2C
$-H^{\oplus}$
$R_2C{=}O$
+
$S(CH_3)_2$


b.
H
CH_2
C_6H_{11}
NH
$\oplus S - O - C$
CH_3
N
C_6H_{11}
$-DCU$
CH_2
$\oplus S$
CH_3
R_2CHOH
$R_2CHOCH_2SCH_3$

Scheme 12.

The diprotected nucleoside is stirred with dry DMSO and toluene (1:1, v:v) to which is added excess dicyclohexylcarbodiimide (DCC). Pyridinium trifluroacetate is used as the proton source . The reaction mixture rapidly turned dark brown, and was allowed to stir overnight at room temperature. Workup consisted of the addition of oxalic acid, followed by water to destroy residual DCC. After dilution with chloroform, and filtration, the mixture was separated into its organic and aqueous layers, and the organic layer washed with aqueous sodium bicarbonate, followed by a wash with water. Removal of solvents *in vacuo*, followed by silica gel chromatography, gave the disilylated 2'- and 3'-ketonucleosides (**72a** and **73a**) in good yield (85 and 63%, respectively). We felt that these yields were sufficient.

The ketonucleosides were characterised by ^{13}C and ^{1}H NMR and IR spectroscopy, as well as UV spectroscopy to ensure that no base modification had occurred. The ^{13}C NMR spectra showed the presence of a new signal at *ca.* 206 ppm, which we assigned to the carbonyl carbon. The proton NMR spectrum showed the loss of one proton from the sugar ring (either the 2'-proton in **72a** or the 3'-proton in **73a**). IR spectroscopy showed the appearance of a new stretch at ca. 1780 cm^{-1}, which we assigned to the newly formed carbonyl. The UV spectrum showed no change from the starting materials, which supports the belief that no base modification had occurred. Interestingly, no evidence of thiomethyl ether formation, a possible side reaction (Scheme 12b) was observed.

These results encouraged us to try the reaction with the other common ribonucleosides. When we carried out the same reaction on disilylated cytidine (**70f** and **71f**) and adenosine (**70e** and **71e**), however, TLC showed several spots, and we were not able to identify any ketonucleoside, as no pure material could be isolated by column chromatography. The column conditions seemed to engender decomposition. This fact, combined with the inconvenience of removing completely the dicyclohexyl urea (DCU) by-product of this reaction, led us to look further. Accordingly, we examined some of the other common "activated DMSO" reagent systems. These included the use of DMSO in conjunction with pyridine-sulphur trioxide[124], acetic anhydride[125], and oxalyl chloride[126]. However, none of these appeared (TLC) to give as good results with the uridine test compounds **70a** and **71a** as did the original Pfitzner-Moffatt oxidation. As we wished to have a very general procedure, it was obviously necessary to try still more reagents.

We then looked into the use of the many chromium-based reagents which have been reported in the literature, many of which are reasonably mild and operate under near-neutral

124. J. R. Parikh, W. von E. Doering, *J. Am. Chem. Soc.*, 89, 5505, (1967).
125. A. J. Mancuso, D. Swern, *Synthesis*, 165, (1981).
126. A. J. Mancuso, D. Swern, S. L. Huang, *J. Org. Chem.*, 43, 2480, (1978).

conditions. We carried out a series of test reactions, again using the disilylated uridines **70a** and **71a** as our models. We performed oxidations with pyridinium chlorochromate[127], and bipyridinium chlorochromate[128], in the presence and absence of molecular sieves[129]. The use of molecular sieves is reported by Herscovici and Antonakis to give increased yields of various carbonyl compounds from their alcohol precursors with shorter reaction times, and have carried out a fairly extensive study of the phenomenon. These authors postulate that a hydride transfer is involved in the reaction, and that the surface of the zeolite assists in this process, possibly by helping align the intermediate in a favourable conformation. It is also possible that molecular sieves assist the reaction by trapping water In any event, we finally settled on pyridinium fluorochromate[130] This crystalline reagent is easily prepared from readily available materials, and operates under mild conditions.

The disilylated uridine nucleoside **71a** was dissolved in methylene chloride and added to a methylene chloride slurry of the oxidising agent (used in excess) After reflux for a few hours, the originally bright orange mixture had turned quite dark. Workup involved simply filtering off the solids, and evaporating the solvent The resulting foam was stirred in ether, refiltered, and evaporated again to give the desired ketonucleoside **73a** as a white foam in good (80%) yield. In many cases, the oxidation was allowed to proceed overnight, with no deleterious effects. This procedure was used for all the remaining oxidations, not only because of its mildness and convenience, but also because of the ease of the workup and product isolation.

The application of this procedure to the remaining partially protected ribonucleosides gave us access to the full range of 2'- and 3'-ketonucleosides **72a-f** and **73a-f**, in their protected forms We chose not to attempt to deprotect the ketonucleosides, due to their inherent instability, as noted above. The conditions required to remove the silyl protecting groups would probably result in extensive decomposition. Certainly the basic conditions used to remove the benzoyl group with which we protected the exocyclic amino groups of the bases would be expected to cause depurination. The results of both the DMSO-DCC procedure (Method A) and the pyridinium fluorochromate procedure (Method B) are reported in Table 1.

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127. E. J. Corey, J. W. Suggs, *Tetrahedron Lett.*, 31, 2647, (1975).
128. F. S. Guziec, F. A. Luzzio, *Synthesis*, 691, (1980).
129. (a) J. Herscovici, K. Antonakis, *J. Chem. Soc. Chem. Comm.*, 561, (1980). (b) J. Herscovici, M.-J. Egron, K. Antonakis, *J. Chem. Soc. Perkin I*, 1967, (1982).
130. M. N. Bhattacharjee, M. K. Chaudhuri, H. S. Dasgupta, N. Roy, *Synthesis*, 588, (1982).

Table 1. Yields and characteristics of oxidation products 72 and 73.

Compound	Method A	Method B	IR(cm^{-1})	λmax(nm)	Rf*	^{13}C(ppm)+
72a	85%	77%	1780	260	.59[a],.25[b]	206.0
73a	63	80	1780	260	.69[a],.36[b]	206.1
72b	78	70	1787	262	.48[c]	206.0
73b	--	85	1787	262	.62[d]	208.2
72c	--	36	1787	278	.40[e]	206.9
73c	--	53	1787	278	.51[f]	209.6
72d	--	74	1787	236	.58[g]	208.5
73d	--	94	1787	236	.63[f]	208.3

*Solvents: a; ether; b; ether:hexane, 1:1; c; ether:EtOH: $CHCl_3$, 71:26:3; d; EtOAc:$CHCl_3$, 4.5 5.5; e; CH_2Cl_2:MeOH, 19:1; f; ether:$CHCl_3$, 1:1; g; methylethyl ketone:MeOH:cyclohexane:$CHCl_3$, 4:.5:4.5:1.

+Referenced to TMS. Run in acetone-d6.

Reduction of the protected ketonucleosides.

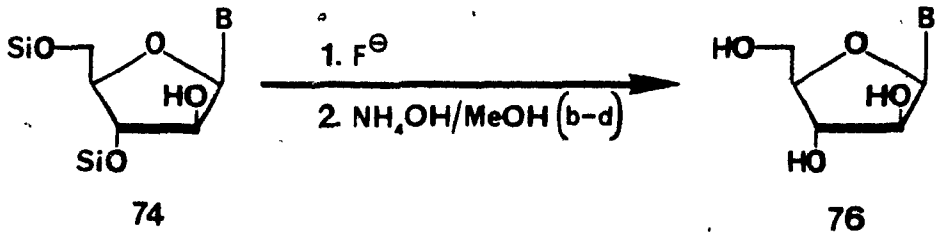
We then wished to carry out the reduction of the carbonyl groups to obtain the desired hydroxyl-inverted nucleosides **74** and **75**. We chose to use Superhydride™ (lithium triethyl borohydride) which is readily available from Aldrich in a variety of solvents. Convenient for our purpose was the 1M solution in THF. The reductions were carried out in THF with an excess of the reducing agent. The reactions went smoothly and were complete within a few hours. TLC analysis in all cases showed that two products had formed, as expected. Workup consisted of simply diluting with chloroform and washing with water. Separation of the organic layer, drying with sodium sulfate, and silica gel chromatography on thick layer plates gave the two closely separated products **70** and **74** or **71** and **75**. The ribonucleoside **70** or **71** was always the slower moving of the two products, but only slightly, and while one development of the analytical plate was normally sufficient, two developments were usually used to ensure complete separation for preparative purposes. The results are collected in Table 2.

Table 2. Yields and characteristics of reduction products 74 and 75.

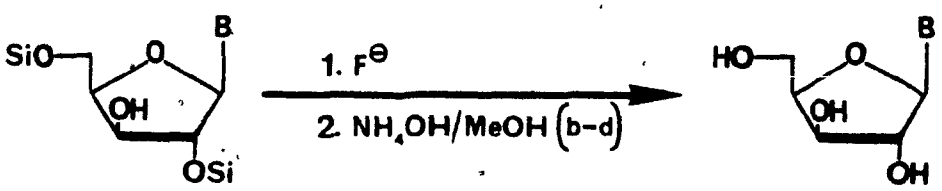
Compound	Yield	MP(°C)	λmax(nm)	Rf*	74,75/70,71
74a	64%	147-150	262	.36[a] .69[b]	64/30
75a	46	212	262	.33[a] .70[b]	46/7
74b	68	152-154	258	.41[c]	10/1
75b	29	105(foam)	258	.44[d]	29/15
74c	10	<90(glass)	277	.36[e]	10/8
75c	31	211	277	.45[e]	31/6
74d	25	184-186	236	.28[e]	25/5
75d	63	115-117	235	.60[f]	63/12

*Solvents: a; ether:hexane, 3:1; b; ether; c; $CHCl_3$:acetone, 9:1, developed twice; d; EtOAc:CH_2Cl_2, 1:3; e; CH_2Cl_2:MeOH, 19:1; f; $CHCl_3$:acetone, 9:1.

The products were characterised by the use of IR spectroscopy to check the disappearance of the carbonyl peak at *ca.* 1780 cm^{-1}. Proton NMR showed the expected nucleoside sugar coupling systems, and signals for the protecting groups while UV spectral analysis again indicated the absence of any unwanted base modification.



74 76



75 77

B as for Scheme 10.

Scheme 13.

To further characterise our reduction products, we deprotected a small sample of each (Scheme 13), for comparison with authentic samples (where available). We found that no sample of any xylonucleoside was readily available, but were able to obtain all the aranucleosides **76a-d**. Deprotection was accomplished by fluoride ion desilylation using tetra-*n*-butylammonium fluoride in THF. After cation exchange on a Na^+ Dowex column, benzoyl groups (when present, compounds **74b-d** and **75b-d**) were removed by hydrolysis with ammonium hydroxide·methanol at 50°C overnight. The fully deprotected compounds were purified by paper chromatography and characterised by NMR, with chemical shifts and coupling constants in accord with those in the literature[131]. UV spectra indicated the complete removal of base protecting groups and lack of other modification at the bases.

Comparison with literature results.

We were interrupted in this work by the publication of two reports along similar lines. In one case[132], Japanese workers prepared the arabino analogues of several 3',5'-di-O-acylated nucleosides. The ketonucleoside was formed by the action of DMSO-trifluroacetic anhydride or DMSO-acetic anhydride on the partially protected nucleosides. Reduction *in situ* using sodium borohydride followed by deacylation with sodium methoxide gave the corresponding aranucleosides (**74**, B= adenine, N^6-benzyladenine, N^2-benzoylguanine, 6-methylthiopurine, 4-amino-pyrollo[2,3-d]pyrimidine, Si replaced with benzoyl). The arabino epimers were isolated in yields of 38-70 %. These workers reported that they were unable to isolate the intermediate ketonucleosides by silica gel chromatography, due to decomposition. They did not report the extent of the decomposition, nor did they give details on the chromatography conditions. This instability is, however, in accordance with the previously mentioned tendency of ketonucleosides, to undergo facile depurination or depyrimidination.

It is especially interesting to compare our results with those of Hansske *et al.*[133] In their first report (Ref. 133a), this group used chromium trioxide:pyridine.acetic anhydride to oxidise 5'-tritylated thymidine, 3',5'-di-O-*t*-butyldimethylsilyluridine (**70a**) and 2',5'-di-O-*t*-butyldimethylsilyladenosine (**70e**), as well as 3',5'-O-(1,1,3,3-tetraisopropyldisilox-1,3-

131. (a) Ara-G, J. B. Chattopadhyaya, C. B. Reese, *Synthesis*, 908 (1978) (b) Ara-U and ara-C, E. Ekiel, M. Remin, E. Darzynkiewicz, D. Shugar, *Biochim Biophys. Acta*, **562**, 177 (1979) (c) Ara-A, M. Kaneko, M Kimura, T. Nishimura, B Shimizu, *Chem. Pharm. Bull.*, **25**, 2482 (1977) (d) Xylo-A, M J. Robins, Y. Fouron, R. Mengel, *J. Org. Chem.*, **39**, 1564 (1974), (e) Xylo-G, W. W. Lee, A P Martinez, L. Goodman, *J. Org. Chem.*, **36**, 842 (1971).

132. N. Sakairi, I. Hirao, Y Zama, Y Ishido, *Nucleosides & Nucleotides*, **2**, 221, (1983).

133. (a) F. Hansske, M. J. Robins, *Tetrahedron Let.*, **24**, 1589, (1983). (b) F. Hansske, D. Madej, M. J. Robins, *Tetrahedron*, **40**, 125, (1984).

and -inosine to the corresponding ketonucleosides. Isolation of the ketonucleosides followed by reduction using sodium borohydride or deuteride gave the ara- or xylonucleosides (or their deuterated analogues) in good (67-88%) yields.

The same group's second report (Ref. 133b) was more complete, including the synthesis of ara-A, -G, -C, and -U (44, B = adenine, guanine, cytosine and uracil) and ara-3'-deoxy-A, as well as xylo-U and -A (45, B = adenine and uracil) and xylothymidine. They noted an interesting problem with their synthesis. When the chromium-based reagent mentioned above was used for the oxidation of 3',5'-di-O-*t*-butyldimethylsilyladenosine (70e), or the 3',5'-diprotected inosine, doubling of peaks was observed in the ^{1}H NMR. This was ascribed to the complexation of chromium ions by the unprotected exocyclic amino group, forming diastereoisomers. In our case, no such doubling was observed in any case. We had difficulty with decomposition when exocyclic amino groups were left unprotected. Thus we used benzoyl protection at the amino groups of adenosine, cytidine and guanosine. It is possible that this protection eliminates chromium complexation. We and Hansske both note that this line doubling problem does not arise with uridine nucleosides, which have no exocyclic amino groups, but whose heterocyclic ring contains an imide-type structure. Nor did they have any such difficulty with cytidine, 2'-deoxyadenosine, or 2',5'-protected adenosines. Hansske *et al.* avoided the complexation problem by switching to Pfitzner-Moffatt oxidation conditions. We feel that the use of amino protection, coupled with chromate oxidation is an improvement, since no chromatography of the somewhat labile ketonucleoside is required, and there is no need to remove the surprisingly persistent DCU which in our case is a byproduct of the Pfitzner-Moffatt conditions, or take precautions against extraneous moisture. Furthermore, N-protection at the ribonucleoside stage allows for further manipulation, and is particularly useful if incorporation of the ara- or xylonucleoside into oligonucleotides is contemplated.

In comparing our results with those of Hansske and coworkers, it is clear that the use of sodium borohydride as the reducing agent is much to be preferred over that of Superhydride^{tm}. We found a relatively poor degree of selectivity for the desired epimers. Our best ratio of inverted hydroxyl:ribo conformation was the 46:7 found for the reduction of 73a. Our poorest was the 10:8 for the reduction of 72c, although this is perhaps not the best example due to the low recovery of product. The results obtained by Hansske *et al.* show, in most cases, about a 10:1 ratio in favor of the desired epimer. In some case the desired epimer was obtained essentially quantitatively. It is quite possible that this difference is related to the steric bulk of the reducing agent. The intermediate formed during the borohydride reduction of carbonyls is thought to involve complex formation between the oxygen of the incipient hydroxyl group and the boron atom of the reducing agent. Clearly, the complex formed between the carbonyl in question and borohydride will be less

sterically congested than that between the carbonyl and triethylborohydride. This may explain the lower yields of "up" (arabino or xylo) epimers using the latter reagent. Crowding between the boron-oxygen complex formed at the reaction site, and the heterocyclic base could slow the reaction giving the desired epimer, allowing the formation of the undesired ribo epimer. Additionally, the relatively slow reduction may permit the reducing agent to act as a base, leading to abstraction of the α-proton and subsequent β-elimination of the base, as previously described. This may explain the relatively low recovered yields of nucleoside in some of our examples.

Conclusions.

In summary, we, simultaneously with others, have outlined useful procedures for the synthesis of ara- and xylonucleosides. These procedures are facile and straightforward, involving readily available reagents, and simple chemical procedures. They should easily be adaptable to the synthesis of nucleotide analogue precursors. We have demonstrated the use of these methods to produce the unnatural epimer at both the 2'- and 3'-positions of all four common ribonucleosides. We are the first, to our knowledge, to make all eight compounds **44** and **45** by a single procedure, although the other workers mentioned above (Ref. 133) could in principle apply their procedure to the synthesis of xylo-G (**45**, B=guanine) and xylo-C (**45**, B=cytosine).

Experimental Section

Materials and methods.

Ribonucleosides were purchased from Boehringer Mannheim Canada (Montreal, Quebec) and used as received. Triisopropylsilyl chloride, *t*-butyldimethylsilyl chloride and Superhydride (1M solution in THF) were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin) DCC was obtained from Aldrich Chemical Co. and melted in a water bath before being added by Pasteur pipette to the reaction flask. All other reagents were purchased as reagent grade from the usual commercial sources. THF was predried over potassium hydroxide pellets and distilled from sodium benzophenone ketyl just before use. DMSO was distilled from calcium hydride after predrying over potassium hydroxide, and stored over 4A molecular sieves. Pyridine was dried by refluxing overnight with phthalic anhydride followed by distillation onto 4A molecular sieves. The protected nucleosides were prepared by published procedures. All reactions were carried out in oven-dried (*ca.* 130^{o}C) glassware and dried in a stream of nitrogen or argon before use. Arabinonucleoside standards were purchased from Sigma Chemical Co.(St. Louis, Mo.), except for ara-G which was prepared by the literature procedure[76]. NMR spectra were acquired on a Varian XL-200 (^{1}H) or Bruker WP-90 (^{13}C) instrument, in deuterated solvents obtained from Merck, Sharp and Dome (Montreal, Quebec) or Aldrich Chemical Co. IR spectra were recorded on a Perkin-Elmer 297, and UV spectra on either a Unicam SP-800 or a Cary 17. Melting points were taken on a Fisher-Johns apparatus and are reported uncorrected. Column chromatography was done using Merck Kieselgel 60, and preparative TLC with Merck Kieselgel 60 GF, 2 mm thick, 20 x 20 cm plates, prepared here, with a maximum loading of 100 mg per plate. Compounds were eluted from the preparative plates using ethanol:ethyl acetate, 3:7. Analytical TLC was done with Merck Kieselgel 60 F plastic-backed plates. Paper chromatography was done on Whatman 1 paper for analytical scale, Whatman 3 MM paper for preparative scale. Cation exchange was done using Na^{+} Dowex ion exchange resin (J. T. Baker Co., Phillipsburg, N.J.).

Oxidations:

Method A.

Preparation of 2'-keto-3',5'-di-O-*t*-butyldimethylsilyluridine (72a).

70a (2.98g, 6.30 mmol) was dissolved in a mixture of toluene (85 ml) and anhydrous DMSO (85 ml). DCC (8.40 g, 42.0 mmol) was added as a liquid, followed by trifluoroacetic acid (0.85 ml, 19.0 mmol) and anhydrous pyridine (1.40 ml, 28.0 mmol). The solution was stirred at room temperature overnight (23 h) during which time it became cloudy and very dark. Oxalic acid (8.00 g, 11.35 mmol) was added and the mixture stirred an additional 30 min. Water was added to destroy the unreacted DCC, resulting in the formation of a large amount of white precipitate. The mixture was diluted with $CHCl_3$ (*ca* 100 ml), filtered, and washed with 5% $NaHCO_3$. The organic layer was washed with water and dried by filtration through magnesium sulphate. The solvent was evaporated and the resulting yellow foam dissolved in ether:hexane, 1:1, and purified on a silica gel column (ether:hexane, 1:1 to 3:1 gradient) to yield **72a** (2.98 g, 85% yield) as an off-white powder. M.p. 179-181°C with decomposition, λmax 260 nm, IR 1780 cm^{-1}, ^{13}C NMR 206.01 ppm.

Preparation of 3'-keto-2',5'-di-O-*t*-butyldimethylsilyluridine (73a).

The reaction was carried out as for the synthesis of **72a**, using 2',5'-di-O-*t*-butyldimethylsilyluridine (2.98 g, 6.4 mmol), toluene (64 ml), DMSO (64 ml), DCC (6.40 g, 31.0 mmol), pyridine (0.96 ml) and trifluoroacetic acid (0.64 ml) After 20 h, the reaction was worked up as above using oxalic acid (6.50 g). The yield after chromatography (ether:hexane, 1:1) was 1.86 g, 63%, of **Va** as a white foam. M. p. 176-179°C, λmax 260 nm, IR 1780cm^{-1}, ^{13}C NMR 206.14.

Preparation of N^4-benzoyl-2'-keto-3',5'-di-O-*t*-butyldimethylsilylcytidine (72b).

As for the synthesis of **72Ia**, using N^4-benzoyl-3',5'-di-O-*t*-butyldimethylsilylcytidine as starting material (0.61 g, 1.00 mmol), toluene and DMSO (10.0 ml each), pyridine (0.30 ml), trifluoroacetic acid (0.20 ml), and 24 h reaction time. The usual workup gave **72b** after chromatography (gradient from $CHCl_3$ to ether:$CHCl_3$:ethanol, 71:26:3) as a white foam, in 78% yield. M. p. 193-195°C with decomposition, softening at *ca.* 180-185°C , λmax 262 nm, IR 1787cm^{-1}, ^{13}C nmr, 206.01 ppm.

Attempts to prepare 2'-keto-3',5'-di-O-*t*-butyldimethylsilylcytidine 72f and 3'-keto-2',5'-di-O-*t*-butyldimethylsilylcytidine 73f.

The reaction conditions described above led to the formation of a mixture of at least four products (TLC). These were impossible to separate by chromatography.

Attempt to prepare 2'-keto-3',5'-di-O-*t*-butyldimethylsilyladenosine 72e and 3'-keto-2',5'-di-O-*t*-butyldimethylsilyladenosine 73e.

The reaction conditions described above were used. In both cases, TLC after workup showed the expected pattern, but on standing, TLC showed rapid decomposition. In addition, TLC after silica gel column chromatography showed evidence of decomposition on the column, and the desired product was not isolated.

Trials of other activated DMSO oxidants.

a. **70a** (1.42 g, 3.00 mmol) was stirred with anhydrous DMSO (10.0 ml) and triethylamine (2.50 g), with the addition of pyridine:SO_3 complex (1.00g)dissolved in DMSO (10.0 ml). After 30 min stirring at room temperature, a further 1.00 g pyridine:SO_3 in 10.0 ml DMSO was added. After stirring overnight the reaction was worked up by addition of oxalic acid, dilution with $CHCl_3$ and extraction, and purified on silica gel to yield 7% (100 mg) of the desired **72a**.

b. **70a** (200 mg, .42 mmol) was dissolved in DMSO (25.0 ml) and acetic anhydride (5.0 ml, 5.0 mmol) was added. After 20 h stirring at room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with aqueous sodium bicarbonate, dried (magnesium sulphate) and concentrated. Purification on TLC plates gave **72a**, in 12.5% yield.

c. Oxalyl chloride (1.00 ml, 11.0 mmol) was dissolved in CH_2Cl_2 (25.0 ml) in a 50 ml round bottom flask fitted with a septum, stirring bar, and drying tube, and cooled in a Dry Ice:isopropanol bath. DMSO (3.00 ml in 3.00 ml CH_2Cl_2) was added *via* syringe over about 2 min. **70a** (200 mg, .42 mmol) was added, dissolved in CH_2Cl_2 (10.0 ml). The reaction mixture was allowed to warm to room temperature overnight. After 20 h, triethylamine (7.00 ml, 50 mmol) was added and the reaction worked up 15 min later, by washing with water. The organic layer was dried and evaporated. TLC of the CH_2Cl_2 solution showed only a faint trace (less than 5%) of the desired product (**72a**).

Studies with other oxidants (chromium-based).

a. **70a** (200 mg, .42 mmol) was added to a suspension of bipyridinium chlorochromate (270 mg,0 .90 mmol) and crushed 4A molecular sieves (500 mg) in CH_2Cl_2 (20.0 ml). The mixture was refluxed overnight, and filtered. Purification on TLC plates (ether:hexane, 1:1) gave **70a** (29 mg, 15%), and **72a** (77 mg, 39%). The same reaction carried out without molecular sieves gave (TLC) a similar product distribution (TLC).

b. **70a** was treated as above with pyridinium chlorochromate (200 mg, 0.93 mmol). TLC analysis (ether:hexane, 3:1) showed similar product distributions as above, in both cases.

Synthesis of pyridinium fluorochromate (PFC).[130]

Chromium trioxide (15.0 g, 150 mmol) was dissolved in water (25.0 ml) in a polyethylene bottle. Hydrogen fluoride (9.40 ml of a 48% aqueous solution, 225 mmol) was added and the solution stirred 5 min. Pyridine (11.3 ml, 150 mmol) was added dropwise over about 10 min, with stirring. The reaction mixture warmed noticeably. The bright orange solution was heated in the water bath for 15 min to redissolve the small amount of precipitate, which had formed, and was then left to stand at room temperature. The mixture was then filtered to yield bright orange crystals, which were dried by blotting with filter paper, then drying *in vacuo* overnight (20.0g, 67%).

Oxidations with PFC.

Preparation of 2'-keto-3',5'-di-O-*t*-butyldimethylsilyluridine (72a).

70a (1.10 g, 2.31 mmol) was dissolved in CH_2Cl_2 (10.0 ml), and added to a suspension of PFC (1.20 g, 6.0 mmol) in CH_2Cl_2 (70.0 ml). The mixture was refluxed 18 h. The mixture turned dark. Filtration led to a yellowish solution, which was evaporated, stirred in ether, refiltered and evaporated, to yield 72a as an off-white powder (850 mg, 77%). This material was identical by TLC, ^1H and ^{13}C nmr, IR and UV, and melting point, to the material prepared by the DMSO-DCC route described above.

Preparation of 3'-keto-2',5'-di-O-*t*-butyldimethylsilyluridine (73a).

71a (1.00 g, 2.10 mmol), was treated as above with PFC (1.00 g, 5.0 mmol). After workup, the desired **73a** was isolated as a white foam (800 mg, 80%), identical chromatographically and spectroscopically to the product of the Moffatt oxidation.

Preparation of N^4-benzoyl-2'-keto-3',5'-di-O-*t*-butyldimethylsilylcytidine (72b).

70b (1.20 g, 2.0 mmol) was dissolved in CH_2Cl_2 (5.00 ml) and added to a suspension of PFC (1.20 g, 6.0 mmol) in CH_2Cl_2 (80.0 ml) and refluxed 20 h. Filtration and evaporation of the solvent gave a foam which was stirred in ether and refiltered. After evaporation of the ether, there was still a slight green color to the resulting foam. It was dissolved in the minimum amount of hot absolute ethanol and recrystallised by addition of water, to yield an off-white powder (840mg, 70%). This material was identical chromatographically and spectroscopically to the material prepared by the Pfitzner-Moffatt procedure (A).

Preparation of N^4-benzoyl-3'-keto-2',5'-di-O-*t*-butyldimethylsilylcytidine (73b).

71b (1.00 g, 1.60 mmol) was dissolved in CH_2Cl_2 (10.0 ml) and added to a suspension of PFC (1.00 g, 5.0 mmol) in CH_2Cl_2 (80.0 ml) and refluxed 20 h. The usual workup gave **73b** as a white powder (0.85 g, 85%). M. p. 172-178°C with decomposition (softens at 163-168°C), λmax 262 nm, ^{13}C NMR 208.23 ppm, IR 1787 cm^{-1}.

Preparation of N^6-benzoyl-2'-keto-3',5'-di-O-*t*-butyldimethylsilyladenosine (72c).

70c (0.80 g, 1.33 mmol) was dissolved in CH_2Cl_2 (20.0 ml) and added to a suspension of PFC (0.80 g, 4.0 mmol) in CH_2Cl_2 (60.0 ml). After refluxing 18 h, the usual workup yielded 72c as a slightly brownish oil (.286 g, 36%). ^{13}C NMR 206.93 ppm, IR 1787 cm^{-1}, λmax 278 nm.

Preparation of N^6-benzoyl-3'-keto-2',5'-di-O-*t*-butyldimethylsilyladenosine (73c).

71c (0.84 g, 1.40 mmol) was dissolved in CH_2Cl_2 (20.0 ml) and added to a suspension of PFC (0.84 g, 4.2 mmol) in CH_2Cl_2 (65.0 ml) and refluxed 16 h. The usual workup and isolation gave **73c** as a light brown foam which quickly collapsed to an oil (.500 g, 59%). ^{13}C NMR 209.58 ppm, IR 1787 cm^{-1}, λmax 278 nm.

Preparation of N^2-benzoyl-2'-keto-3',5'-di-O-triisopropylsilylguanosine (72d).

70d (1.50 g, 2.14 mmol) was treated with PFC (1.50g) in the usual way. After reflux for 48 h, TLC (methylethyl ketone:cyclohexane:$CHCl_3$, 4.5:4.5:1) showed reaction was about 75% complete. A further 1.00 g PFC was added and reflux continued 24h. The usual workup yielded **72d** as a light brown foam (1.11 g, 74%). M. p. 175-180°C, (softens at 165-170°C), ^{13}C NMR 208.49 ppm, IR 1787 cm^{-1}, λ max 260 nm.

Preparation of N^2-benzoyl-3'-keto-2',5'-di-O-triisopropylsilylguanosine (73d).

71d (2.00 g, 2.86 mmol) was treated with PFC (2.00 g, 10 mmol) as for **72d.** Workup yielded **73d** as a light brown foam (1.87 g, 94%). M. p. 132-140°C with decomposition, ^{13}C NMR 208.29, IR 1787 cm^{-1}, λ max 260 nm.

Reductions of products 72 and 73 to give 70, 74 and 71, 75 mixtures.

Reduction of 72a to give 3',5'-di-O-*t*-butyldimethylsilyluridine (70a) and 3',5'-di-O-*t*-butyldimethylsilylarabinouridine (74a).

72a (1.42 g, 3 mmol) was dissolved in anhydrous THF (150 ml). Superhydride^{tm} (6.50 ml 1 M THF solution, 6.50 mmol) was added and the solution stirred 3 h at room temperature. The yellowish solution was partitioned between water and $CHCl_3$, and the organic layer separated and dried with magnesium sulphate. Concentration and purification on thick layer plates ($CHCl_3$:acetone, 9:1, 2 developments) yielded **70a** (.420 g, 30%) and **74a** (.844 g, 64%). **74a** was isolated as a white foam, m. p. 147-150°C, max 262 nm. ^1H NMR ($CDCl_3$) 6.09 (m, 1, H1'), .88 (m, 18, -$C(CH_3)_3$), .11 (m, 12, -Si-CH_3). Ribonucleoside products for this and subsequent reactions were identified by comparison with standards (^1H NMR, UV, Rf).

Reduction of 73a to give 2',5'-di-O-*t*-butyldimethylsilyluridine (71a) and 2',5'-di-O-*t*-butyldimethylsilylxylouridine (76a).

73a (1.42 g, 3.00 mmol) was dissolved in anhydrous THF (150 ml) and Superhydride^{tm} (7.00 ml, 7.00 mmol) was added. The reaction was stirred at room temperature 3 h, then worked up as above. The product, a yellow foam, was purified on plates (carbon tetrachloride:acetone, 7.5:2.5, two developments) to give the desired **75a** (653 mg, 46%) and **71a** (99 mg, 7%). **75a** was a

white foam, m. p. 212°C, λmax 262 nm, ^{1}H NMR ($CDCl_3$) 5.95 (d, 1, H1'), .95 (m, 18, -$C(CH_3)_3$), .08 (m, 12, -Si-CH_3)).

Reduction of 72b to give N^4-benzoyl-3',5'-di-O-*t*-butyldimethylsilylcytidine (70b) and N^4-benzoyl-3',5'-di-O-*t*-butyldimethylsilylarabinocytidine (74b).

72b (150 mg, 0.25 mmol) was treated as above with the reducing agent. The usual workup led to **74b** (100 mg, 68 %) as a yellowish foam, m p. undefined, λ max 258 nm, ^{1}H NMR ($CDCl_3$) 6.25 (d, 1, H1'), .95 (m, 18, -$CH(CH_3)_3$), 05 (m, 12, -Si-CH_3), and a small amount of **70c** (10 mg), after purification on preparative plates ($CHCl_3$.acetone, 9:1, two developments)

Reduction of 73b to give N^4-benzoyl-2',5'-di-O-*t*-butyldimethylsilylcytidine (71b) and N^4-benzoyl-2',5'-di-O-*t*-butyldimethylsilylxylocytidine (75b).

73b (450 mg, 0.95 mmol) was dissolved in anhydrous THF (30.0 ml) to which was added the reducing agent (3.20 ml, 3.20 mmol). After stirring 3 h at room temperature, the reaction was worked up as usual. Purification on plates (EtOAc:$CHCl_3$, 25:75) gave **71b** as a white foam (55 mg, 12%) and **75b** (115 mg, 26%), also as a white foam, m. p. 105°C, λmax 258 nm, ^{1}H NMR ($CDCl_3$) 5.75 (bs, 1, H1'), .90 (d, 18, $C(CH_3)_3$).

Reduction of 72c to give N^6-benzoyl-3',5'-di-O-*t*-butyldimethylsilyladenosine (70c) and N^6-benzoyl-3',5'-di-O-*t*-butyldimethylsilylarabinoadenosine (74c).

72c (500 mg, 0.83 mmol) was dissolved in anhydrous THF and treated as above with Superhydride^{tm} (2.00 ml, 2.00 mmol) for 2 hours at room temperature. The usual workup and purification on plates (CH_2Cl_2:MeOH, 9.5:.5, developed twice) led to the isolation of **70c** (40 mg, 8%) as a white foam and **74c** (49 mg, 10 %) as a colourless glass, m. p. less than 90°C, λmax 277 nm, ^{1}H NMR ($CDCl_3$) 6.44 (bs, 1, H1'), .89 (bs, 18, -$C(CH_3)_3$), .16 (s, 12, -Si-CH_3).

Reduction of 73c to give N^6-benzoyl-2',5'-di-O-*t*-butyldimethylsilyladenosine (71c) and N^6-benzoyl-2',5'-di-O-*t*-butyldimethylsilylxyloadenosine (75c).

73c (270 mg, 0.45 mmol) was treated as usual with the reducing agent (1.10 ml, 1.10 mmol) for two hours. Purification on plates (CH_2Cl_2:MeOH, 9.5:.5) led to the isolation of **71c** (16 mg, 5%) as a foam, and **75c** (85 mg, 31%) also as a foam, m. p. 211°C, λmax 277 nm, ^{1}H NMR ($CDCl_3$) 5.95 (bs, 1, H1'), .90 (bs, 18, -$C(CH_3)_3$), .10 (bs, 12, -Si-CH_3)).

Reduction of 73d to give N^2-benzoyl-2',5'-di-O-triisopropylsilylguanosine (71d) and N^2-benzoyl-2',5'-di-O-triisopropylsilylxyloguanosine (75d).

73d (550 mg, 0.80 mmol) was dissolved in anhydrous THF (25.0 ml) and treated with the reducing agent (2.00 ml, 2.00 mmol) for 2 h, at room temperature. The usual workup and purification on plates ($CHCl_3$:acetone, 9:1, developed twice) gave **71d** (65 mg, 12%) and **75d** (344 mg, 63%) as a yellow foam after evaporation, m. p. 115-117°C, max 260 nm, ^{1}H NMR ($CDCl_3$), 5.90 (bs, 1, H1'), 1.01 (m, *ca.* 42, -C(CH_3)$_3$).

Deprotection of the reduction products 74 and 75.

All of the xylo- and arabinonucleosides were deprotected. A small sample (50 mg) of each was desilylated by reaction with tetra-*n*-butylammonium fluoride (1.00 ml of a 0.1 M THF solution) for two hours in a plastic test tube. The solvent was evaporated, and the resulting oil was taken up in water (1 ml). This was applied to a Dowex Na^+ ion exchange column (25 ml), and eluted slowly with water (30 ml). The water was removed by lyopholisation and those nucleosides bearing benzoyl groups were treated with ammonium hydroxide:MeOH, 4:1 (5 ml) at 50°C overnight in a polypropylene test tube sealed with a rubber septum. The solvent was lyopholised and all fully deprotected nucleosides were dissolved in water, applied to Whatman 3 MM chromatography paper (10 mg per sheet) and developed with isopropanol:water·ammonium hydroxide, 8·1:1. The chromatograms were visualised by UV shadowing with a fluorescent TLC plate, and the UV-absorbing bands cut out and eluted with water. The fully deprotected nucleosides were then characterised by proton NMR and UV spectroscopy. The values are collected in Tables 3 and 4, and are in good agreement with literature values. In addition, the arabinonucleosides were compared chromatographically with standards purchased from Sigma, or (ara-G) prepared in this laboratory by another procedure[76].

Table 3. Characteristics of deprotected ara- and xylonucleosides (44a-d and 45a-d).

Compound	Rf*	λ max(nm)	MP(°C)
44a	.60	262	d>225
45a	.58	262	d>225
44b	.61	270	d190-210
45b	.57	270	d>200
44c	.64	258	d>200
45c	.73	258	d>200
44d	.57	252	d>225
45d	.63	252	d>225

*On paper, using *i*-PrOH:H_2O:NH_4OH, 7:2:1.

Table 4. ^1H chemical shifts of deprotected ara- and xylonucleosides 44 and 45.

i.Sugar protons.

Compound	H-1'($J_{1',2'}$)	H-2'($J_{2',3'}$)	H-3'($J_{3',4'}$)	H-4'	H-5'
44a	5.96(8.1)	3.99(3.5)	3.96(N.R.)	3.69	3.57
45a	5.85(0.5)	3.92(N.R.)	3 92(N.R.)	4.06	3.66
44b	6.02(4.0)	3.95(8.0)	3 85(7.0)	3.71	3.56
45b	5 61(N.R)	3.86(N.R.)	3 86(N.R.)	4.05	3.50
44c	6. 25(3.6)	4.14(N R.)	3.74(N.R.)	3.62	3.54
45c	5.8 5(2.0)	4.29(1.5)	4.02(N.R.)	4.13	3.75
44d	5.90 (4.0)	4.02(N.R.)	4.02(N.R.)	3.71	3.60
45d	5.65(1.5)	4.19(N.R.)	4.00(N.R.)	4.10	3.45

ii. Base protons.

Compound	H-5($J_{5',6'}$)	H-6	Compound	H-2	H-8
44a	7.69(8.1)	5.28	**44c**	8.17	8.10
45a	7.74(8.0)	5.59	**45c**	8.25	8.12
44b	7.55(7.4)	5.67	**44d**	7.71	6.60
45b	7.75(7.5)	5.50	**45d**	7.83	6.59

*Shifts given in ppm from TMS; coupling constants given in Hz;H- 5' values given as the center of multiplet; N.R.=not resolved. Run in dmso-d6.

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Chapter 2.

Synthesis of Silyl Nucleotide Analogues

Introduction.

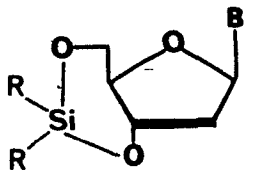
In the general introduction to this thesis, a brief overview of the area of nucleoside and nucleotide analogues was presented. After discussion of several base- and sugar-modified analogues, some of the more prominent linkage-modified nucleotides were described. A wide range of such molecules has been made, with the phosphodiester group replaced by such groups as phosphorothioate, carbonate, carbamate, phosphoramidate, and alkyl- or arylphosphonate. Recently, a new synthesis of a 3'-O-5'-N-carbamate-linked hexathymidylic acid analogue was reported[134].

As mentioned in the general introduction, we have embarked on the synthesis of a novel type of nucleotide analogue, in which the phosphodiester link of the natural nucleotide is replaced with a silyl ether connection. The object was to produce a class of analogues which were neutral, but which still retained some similarity to the natural form. Like phosphorus, silicon is tetrahedral, with the Si-O bond length of approximately 163 pm as compared to about 155 pm for the P-O bond of natural nucleotides (for a neutral nucleotide phosphotriester, this bond length is about 157 pm[135]). In addition, the molecules should be relatively easily synthesised, and stable under at least mildly acidic and basic conditions, in order to facilitate their handling.

Silicon has been fairly widely used in the nucleotide field as a basis for protecting groups. Aside from the use of *t*-butyldimethylsilyl ethers which were described in the previous chapter, a wide variety of other silicon ether protecting groups have been described, varying in the nature of the substituents at silicon. The principal difference is in the steric bulk of those substituents. In general, larger alkyl or aryl groups promote greater hydrolytic stability of the resulting ether, at the price of greater difficulty in forming that ether. Fortunately, in most cases both primary and secondary hydroxyls can be silylated by most silyl protecting groups, and conditions can be used which allow relatively selective reaction at the primary hydroxyl. This area has recently been reviewed by Lalonde and Chan[136]. The particular advantage of silyl protecting groups is the stability of the resulting ether. These are stable to many of the common reagents of organic

134. J. M. Coull, D. V. Carlson, H. L. Weith, *Tetrahedron Lett.*, **28**, 745 (1987).
135. R. G. Brennan, N. S. Kondo, M. Sundaralingam, *J. Am. Chem. Soc.*, **106**, 5671 (1984).
136. M. Lalonde, T. H. Chan, *Synthesis*, 817 (1985).

synthesis, yet are easily removed with fluoride ion. This stability made us consider the synthesis of silyl-linked nucleotide analogues.



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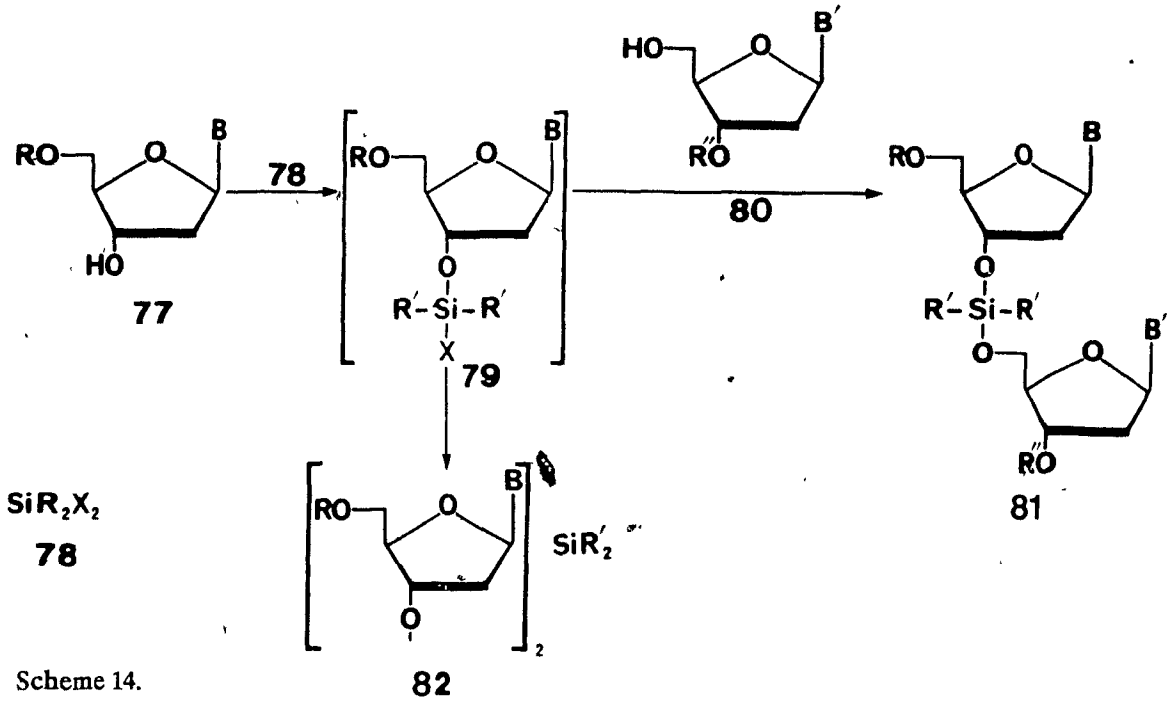
At the time we undertook this work, no report had come to our attention of any such silicon-linked nucleotide analogue. Since that date, only two reports, of silyl analogues (**76**) of some 3',5'-cyclic phosphates, have appeared[137]. Other than our own work, we are unaware of any linear silyl nucleotide synthesis. We were interested in whether such molecules could be synthesised, the effect of various substitutions at silicon on the stability of the products, and the nature of those final products; in particular, whether they exhibited the base-stacking characteristics known for natural nucleotides, and if they could form duplexes with the natural molecules. We found that we could indeed synthesise the desired 3',5'-linked silyl nucleotides with a variety of substituents at silicon. We were able, in addition, to make oligomers up to six units long, using the common protecting groups of oligonucleotide synthesis. These sequences could be deprotected without too much difficulty, provided that a sufficiently stable silyl link was used. We used thymidine as a representative pyrimidine nucleoside, and 2'-deoxyadenosine as an example of a purine nucleoside. These present all of the protection problems commonly found in nucleotide synthesis, and the product nucleotides would be complementary to one another.

Synthetic Approach.

We used an approach (Scheme 14) similar to that used in the solution phase synthesis of oligonucleotides. A solution of a suitably protected nucleoside **77** was added dropwise to a solution containing the silylating agent and excess base. After a time during which the intermediate silylated nucleoside **79** would form, the second partially protected nucleoside **80** was added, also in solution, and the reaction mixture left to stir. We hoped that reaction at the 3'-hydroxyl of the first nucleoside would be fairly selective, that is that the production of the undesired 3',3'-linked dimer **82**

137. (a) K. Furusawa, T. Katsura, *Tetrahedron Lett.*, **26**, 887 (1985). (b) K. Furusawa, T. Katsura, T. Sakai, K. Tsuda, *Nucl. Acids Res. Symp. Ser.*, **16**, 85 (1985).

would be small. Workup, chromatography and deprotection would lead to the desired dimer **81**. Use of suitable protecting groups R and R' would allow for chain extension in either direction if this was desired. Of course, in a reaction where the ratio 77:78:80 is 1:1:1, production of 1 mole of **82** will lead to the production of the same amount of the other possible symmetrically-linked dimer **83**, by reaction of **80** with the residual silylating agent. Clearly, three problems confronted us.



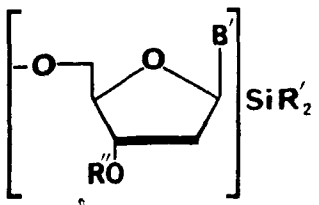
Scheme 14.

First, a silylating agent **78** was needed which would undergo this reaction, and form a silyl ether which would be stable to both workup and deprotection conditions. There are a great many silylating agents commercially available, and our initial selection of such an agent was dictated by this availability. Most of these reagents are dichlorosilanes (**78**, X = Cl) which are sufficiently reactive for most purposes, while still being stable to long-term storage. As will be seen later, leaving groups other than chloride may also be used.

Second, the reaction conditions themselves should allow reasonably selective coupling leading to the desired asymmetrically linked product **81**, minimising or eliminating production of the symmetrically linked byproducts **82** and **83** (*vide infra*).

Third, the protecting groups R and R" must be removed independently of one another, under conditions compatible with the integrity of the silyl link. It would obviously be useful if the partially protected nucleosides themselves were easily synthesised.

Our initial experiments revolved around the synthesis of a 3',5'-linked thymidine dimer **84**. We chose to use dichlorodiphenylsilane as the silylating agent, 4,4'-dimethoxytrityl (DMT) as the 5'-protecting group, and 4-monomethoxytrityl (MMT) as the 3'-protecting group. This would allow us to selectively remove the 5'-protecting group using zinc bromide[138], and at the end of the synthesis the whole molecule could be deprotected in one step with acid. We used thymidine as it presents the minimum of protection problems.



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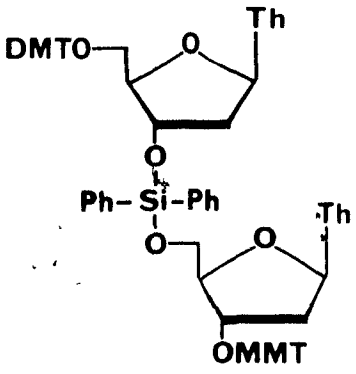

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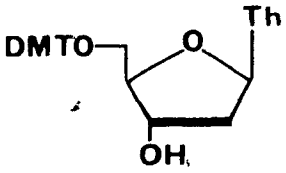

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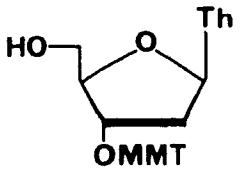

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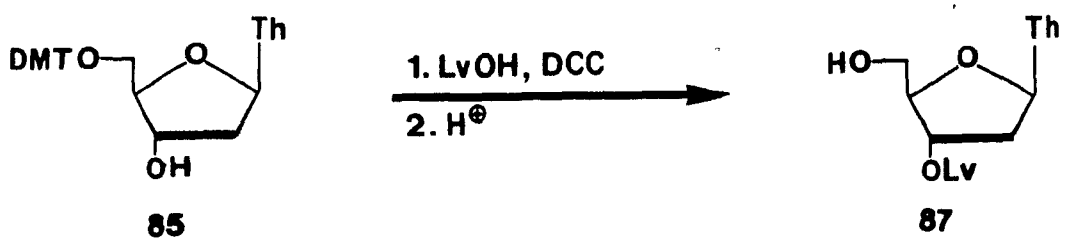
Addition of a DMF solution of the 5'-protected thymidine **85** to a DMF solution of the silylating agent and imidazole at room temperature, followed by addition of the 3'-protected nucleoside **86**, led to a mixture of products. All were tritylated. From the colour of the TLC spots on spraying with dilute perchloric acid, it seemed that two of the three products bore dimethoxytrityl groups (giving rise to bright orange spots), and one a monomethoxytrityl group (strong yellow spot). After column chromatography, we were able to isolate the desired 3',5'-linked dimer (**84**) in only 29% yield. This was identified by its ^{1}H NMR. The other products were identified as the 3',3'- and 5',5'-linked dimers **82** and **83** (B=B'=thymine, R=DMT, R'=phenyl, R"=MMT), based on their ^{1}H NMR. Attempts to deprotect the 3',5'-linked dimer **84** were unsuccessful. Treatment with acetic acid, or 3% dichloro- or trichloroacetic acid in methylene chloride under conditions which would lead to removal of the both the 3'-monomethoxytrityl and the 5'-dimethoxytrityl protecting groups led to decomposition and the production of thymidine. Obviously, both our protecting group strategy and reaction conditions would have to be changed.

Fortunately, a multitude of hydroxyl protecting groups have been developed for nucleotide synthesis. We wished to retain the dimethoxytrityl group at the 5'-position since it provides a good

138. M. D. Matteucci, M. H. Caruthers, *Tetrahedron Lett.*, **21**, 3243 (1980).

marker on TLC, and is easily removed with mild acid. In addition, 5'-dimethoxytritylated nucleosides are easily synthesised in good yield. We required a good 3'-protecting group. A number were ruled out due to incompatibility with our procedure. Most of the ether-type protecting groups are removed under conditions incompatible with our silyl link, or are not stable under detritylation conditions. The use of silyl protection, such as *t*-butyldimethylsilyl or triisopropylsilyl, was obviously also ruled out, since the deprotection conditions would certainly destroy the silyl link. We wanted a protecting group which was easily introduced, and stable to acid, but removed under mild conditions. The levulinyl group[139] satisfies these requirements. It is introduced by a simple esterification procedure, and removed rapidly using hydrazine in pyridine:acetic acid solution. The methyl moiety of this group provides a convenient marker in the ^{1}H NMR. We exposed compound **84** to the delevulination conditions described in the literature, and found it was stable, provided the reaction was not allowed to run more than a few minutes. The required 3'-levulinylthymidine **87** is easily prepared from **85** by esterification with levulinic acid in dioxane (Scheme 15), in the presence of DCC and a catalytic amount of N,N-dimethylaminopyridine No detritylation was observed, either visually (there was no orange colour to the reaction) or on TLC Removal of the dimethoxytrityl group with 80% aqueous acetic acid gives the desired **87** in good (90% for the first step, 62% for the second) yield after crystallisation from toluene.

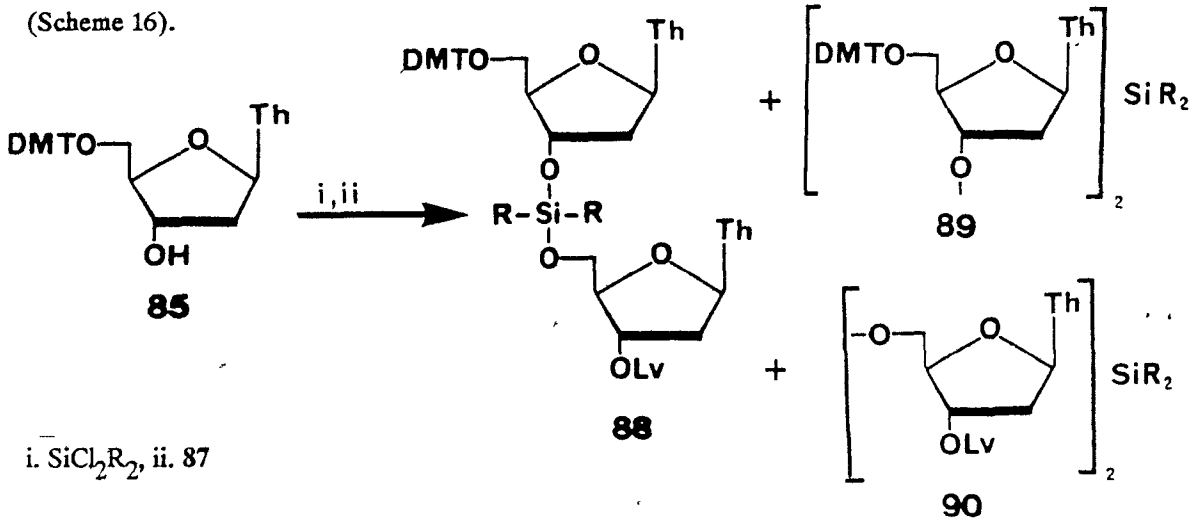


Scheme 15.

We then turned to the problem of finding new coupling conditions. We obviously had a problem with the reaction of the nucleoside 3'-silylchloride (**79**, X=Cl) with the 3'-hydroxyl of another molecule of **77** (Scheme 14). In other words, we wished to use conditions under which the free silylating agent was much more reactive than intermediate **79**. We varied the temperature and solvent, using DMF (known to be a good solvent for silylation reactions, especially in the presence of

139. J. H. van Boom, P. M. J. Burgers, *Tetrahedron Lett.*, 4875 (1976).

imidazole,both for hydroxyls in general[140] and nucleosides in particular[141]) and THF, at room temperature and reduced temperature, with either pyridine or imidazole as base, with or without silver nitrate assistance[142]. We used 87 as the second nucleoside, and the reactions were carried out essentially as already described, and monitored by TLC. We found our best results were obtained using THF as solvent and pyridine as base, and carrying out the reaction at -78°C using an isopropanol:Dry Ice bath. This gave us the desired **88b** in 53% yield, along with **89b** in 23% yield (Scheme 16).



i. $SiCl_2R_2$, ii. 87

R = a, Et; b, phenyl; c, *t*-Bu, phenyl; d, *t*-Bu, Me; e, *i*-Pr; f, Me; g, $(CH_2)_4$; h, *t*-Bu.

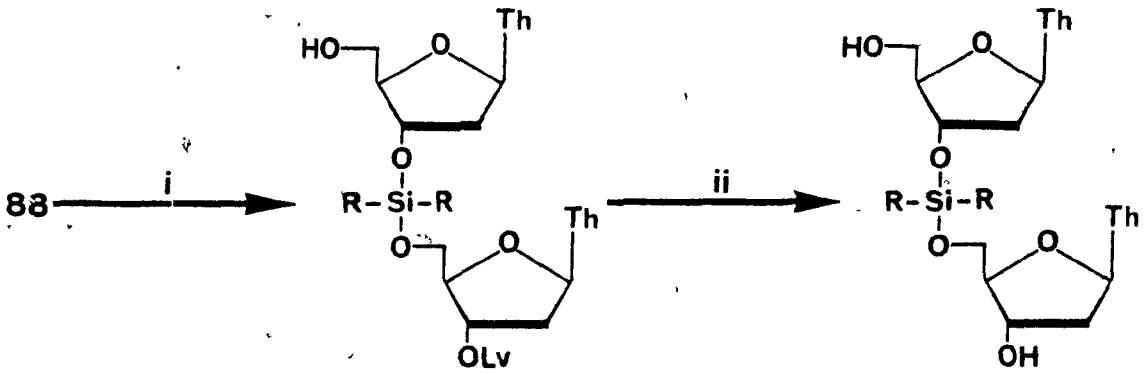
Scheme 16.

We followed the progress of the reaction by TLC. This shows the appearance of two dimethoxytritylated materials after the first nucleoside has been added. The faster of the two disappears on addition of the second nucleoside. The more polar material appears unchanged after the addition of the second nucleoside. This more polar material was identified by ^1H NMR as the symmetrically linked **89b**. On addition of the second nucleoside, the faster-moving tritylated material is consumed, and a new, more polar tritylated material appears. This is the asymmetrically-linked dimer **88b**. There is also a new, nontritylated material, close to the baseline. This is identified by its proton NMR as the 5',5'-linked dimer **90b**. The recovered yield of this material is usually very low. Thus, chromatographically, we find that **89b** is the least polar compound, followed by **88b**, and **90b** appears last on the TLC. This order was followed in all but one of the syntheses in this chapter. Obviously, this makes purification somewhat difficult as the desired compound is found between the two major byproducts.

140. A. R. Bassindale, T. Stout, *Tetrahedron Lett.*, **26**, 3403 (1985).
141. K. K. Ogilvie, A. L. Schifman, C. L. Penny, *Can. J. Chem.*, **57**, 2230 (1978).
142. G. H. Hakimelahi, Z. A. Proba, K. K. Ogilvie, *Tetrahedron Lett.*, **22**, 4775 (1981).

Deprotection of the Dimer.

We were able to remove the dimethoxytrityl group of **88b** in good (78%) yield using zinc bromide in anhydrous nitromethane (Scheme 17). This very mild procedure left the levulinyl group untouched, and there was no evidence of decomposition of the diphenylsilyl link. We also attempted to detritylate using catalytic phase transfer hydrogenation[143], which involves refluxing the substrate in an ethanol:cyclohexene mixture in the presence of a catalytic amount of palladium oxide. Cyclohexene acts as the hydrogen source. This procedure has been used in this laboratory for the removal of benzyl and dimethoxytrityl protecting groups. In the present case, however, detritylation was accompanied by degradation to thymidine and levulinyl thymidine, as evidenced by TLC. There was no indication that the detritylation was any more rapid than the desilylation (i.e. the reaction time was not the problem) so this procedure was abandoned.



i. $ZnBr_2/NO_2CH_3$, ii.Hydrazine (pyridine:acetic acid, 3:2)
Scheme 17. R = a, Et; b, phenyl; c, *t*-Bu, phenyl; d, *t*-Bu, Me; e, *i*-Pr; f, Me; g, $(CH_2)_4$; h, *t*-Bu.

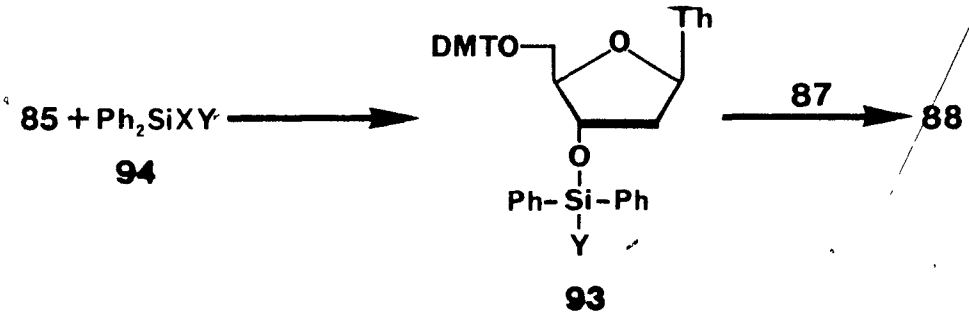
It was observed that treatment of *t*-butyldimethylsilyl-protected nucleosides (70 and 71, B = uracil) under these conditions led to desilylation. To our knowledge, this is the first report of this reaction being used to remove the *t*-butyldimethylsilyl protecting group, although it is not likely that it would be of much synthetic utility as fluoride ion is sufficiently mild and selective for most purposes. Hydrogenolysis of trimethylsilyl ethers is also known[144]. Detritylation of 3',5'-di-O-dimethoxytritylthymidine under these conditions showed no selectivity.

143. (a) I. D. Entwistle, R. A. W. Johnstone, T. J. Povall, *J. Chem. Soc. Perkin I*, 1300 (1975) (b) G. M. Anantharamaiah, K. M. Sivanandaiah, ibid., 490 (1977).
144. A. Holt, A. W. P. Jarvie, J. J. Mallabar, *J. Organometallic Chem.*, 59, 141 (1973).

The levulinyl group was removed (Scheme 17) to give **92b** in 67% yield by treatment of the partially protected dimer **91b** with hydrazine in pyridine:acetic acid (3:2)[139]. The reaction was clean if the time was kept short. After about 3 minutes, deprotection was complete (TLC). Within one or two more minutes, degradation products began to appear on the TLC. Thus it was necessary to stop the reaction quickly, by addition of acetyl acetone to consume unreacted hydrazine, and aqueous workup. This showed the sensitivity of the diphenylsilyl link to basic conditions.

Use of Other Silylating Agents.

We hoped to improve the yields by using a differentially functionalised silylating agent (Scheme 18). We reasoned that if one of the chlorine atoms attached to silicon was replaced by some other good leaving group which either reacted at a different rate or under different conditions than does the parent chloride, we would gain some selectivity in the coupling reaction This is to say, **88** would form so much more rapidly than **89**, due to the different rates of substitution of the two leaving groups, that we would greatly reduce or even eliminate the production of **89** (and thus of **90**). This would both enhance our yields, and ease purification. The reader will note the similarity between this approach, and the phosphoramidite route of natural oligonucleotide synthesis mentioned in the general introduction.



X=Cl, Y=N(Me)$_2$

Scheme 18.

To this end, we obtained the commercially available N,N-dimethylaminodiphenylsilyl chloride **94** (R=phenyl, X=Cl, Y=NMe$_2$). The addition of the first nucleoside **85** was carried out under basic conditions, using pyridine, triethylamine, or diisopropylethylamine as the base. It was hoped that the intermediate nucleoside silylamine **93** (Y=NMe$_2$) could be isolated. This was not the case. After basic workup, ^{1}H NMR showed no evidence of this product. The use of the weak acid 1H-tetrazole to protonate the silylamine *in situ*, followed by addition of **87** in solution, led to

none of the desired dimer. Rather, the only tritylated product observed was the 3',3'-linked dimer **89**. It would seem that even under these basic conditions, the silylamine is a sufficiently strong base to be protonated and act as good leaving group. The reaction of silylamines with hydroxyl groups is known to be promoted by both acidic and basic conditions[145]. On the other hand, it is possible that protonation of the silylamine is followed by nucleophilic displacement by chloride ion to form the nucleoside silyl chloride, which would then react as in Scheme 14. We attempted without success to synthesise other dialkylaminosilyl chlorides, and so this area of the work was discontinued.

We also wished to examine the use of silylating agents with other alkyl or aryl substitutions at silicon. As mentioned above, there are many alkyl- and aryl-substituted dichlorosilanes commercially available. We felt that increasing the steric bulk around the silicon atom might increase its stability to hydrolysis. In protecting group chemistry, varying the size of the substituents at silicon has a large effect on the stability of the resulting silyl ethers. For example, it is well known that trimethylsilyl ethers are cleaved under fairly mild conditions, under which *t*-butyldimethylsilyl ethers are stable and *t*-butyldiphenylsilyl ethers are yet more stable. Thus we felt that we might improve the stability of our nucleotide analogues by the use of more hindered silylating agents. Further, we felt that more hindered silyl chlorides might react more selectively; that is, **79** might show a sufficiently large propensity to react with the primary alcohol of **80** rather than with the secondary alcohol of **77**, so that our yields might be improved.

We were able to obtain a range of dichlorosilanes bearing a variety of groups. We carried out the coupling reaction using the same conditions as before (Scheme 16), THF as solvent and pyridine as base, at Dry Ice temperatures, with these silylating agents. The yields are collected in Table 6. At the same time, we synthesised the symmetrically linked **89a-e** and **90a-e**, as standards for chromatography. It will be noted that the less hindered reagent diethyldichlorosilane gave a very low yields of the desired products **88a**. This seemed quite unstable to column purification. TLC of the attempted syntheses of **88f,g** showed traces of the desired product, but these could not be isolated by silica gel column chromatography.

145. R. Fessenden, J. S. Fessenden, *Chem. Revs.*, **61**, 361 (1961).

Table 5. Characteristics of 3',5'-silyl linked thymidine dimers **88**, **91** and **92**.

Compound	Yield (%)	M.P. (°C)	U.V. (λmax)	Rf*
88a	18.5	105-110	268	.45[a],.27[b],.54[c]
88b	53	113-117	265	.45, .16, .28
88c i.	61+	90-93	266	.45, .31, .45
ii.		103-110	266	.36, .16, .32
88d i.	68+	103-108	268	.31, .27, .48
ii.		106-110	268	.45, .22, .32
88e	37	foam	266	.48, .27, .47
91b	78	91-93	264	.38, .11, .14
91c	47	106-109	266	.34, .11, .15
91e	75	foam	266	.20, .11, .19
92b	67	106-109	265	.25, .03. 06
92c	40	133-140	265	.11, .04, .09
92e	64	104-108	266	.07, .03, .08

*TLC solvents: a, CH_2Cl_2:MeOH, 9:1; b, ether:CH_2Cl_2:EtOH, 71:26:3; c, EtOAc. +Yield for both diastereoisomers.

Table 6. Characteristics of 3',3'-silyl linked thymidine dimers **89**.

Compound	Yield (%)	M.P. (°C)	U.V. (λmax)	Rf*
89a	70	115-118	268	.60[a],.54[b],.76[c]
89b	47	135-140	270	.39, .46, .79
89c	52	130-135	268	.49, .57, .77
89d	62	121-126	270	.63, .53, .74
89e	36	112-115	268	.45, .51, .75
89f	22	115-122	268	.38, .46, .72
89g	60	oil	270	.46, .48, .36

*Same solvents as Table 5.

Table 7. Characteristics of 5',5'-linked thymidine dimers.

Compound	Yield (%)	M.P. (^{o}C)	U.V. (λmax)	Rf*
90a	84	oil	264	.53[a],.09[b],.20[c]
90b	8	<90,	270	.63, .53, .74
90c	73	<80,	266	.49, .11, .19
90d	31	gum	266	.38, .10, .18
90e	35	oil	264	.37, 11, .17
90f	30	foam	266	.42, .11, .11
90g	26	gum	265	.30, .07, .14

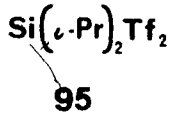
*Same solvents as Table 5.

Some further comment on the synthesis of the symmetrically linked dimers is probably in order. It will be noted that the yields of 5',5'-linked dimers **90** are consistently lower than those of the corresponding 3',3'-linked dimers **89**. In all cases, the reaction mixtures are clean by TLC, showing only the symmetrically linked dimer, and a trace of slightly faster-moving material, presumably the nucleoside silanol resulting from the slight excess of silylating agent used. We speculate that the low yield is due to degradation on the column. Since the symmetrically-linked dimers were not the focus of our research, no attempt was made to optimise their synthesis.

The most hindered reagent, di-*t*-butyldichlorosilane, gives none of the desired 3',5'-linked dimer, even at higher temperatures. In fact, even extended reaction times and the use of silver nitrate to enhance reaction gave none of the product **88h** It may be that the silylating agent is too hindered. The use of the more reactive silyl triflate (in methylene chloride, *vide infra*) did not give any better result In both cases, TLC analysis showed the formation of a single faster-moving tritylated material, apparently analogous to the fast-moving material formed in the silylations described above. In these present reactions, however, no new products formed on addition of the second nucleoside **87**, nor did this second nucleoside appear to be consumed. This was disappointing, since we had expected that the di-*t*-butylsilyl-linked analogue might show the greatest resistance to hydrolysis, and had hoped that it might give more selectivity in the formation of the asymmetrically-linked dimer **88**.

The yields of the analogues bearing *t*-butylphenyl and *t*-butylmethylsilyl links (**88e** and **f**, respectively) were quite respectable (61 and 68%). On the other hand, they are produced as a pair of diastereoisomers, being differentially substituted at silicon. Unlike normal nucleotide synthesis, the present synthesis does not remove the diastereoisomerism at the deprotection step. The diastereoisomers may be separated, as implied in Table 5. This leads to either a loss of material (if the diastereoisomers are separated and only one used in subsequent couplings) or difficulty in

purification at later stages (if both diastereoisomers are used). We felt that this should be avoided. But we were unable to prepare the di-*t*-butylsilyl-linked dimer (*vide supra*) and the diphenylsilyl-linked dimer was somewhat sensitive to acid and base. We felt that a diisopropylsilyl ether link would be a reasonable compromise between reactivity and stability. Unfortunately, the appropriate dichlorosilane is not commercially available. We were, however, able to find a commercial source for the corresponding triflate.



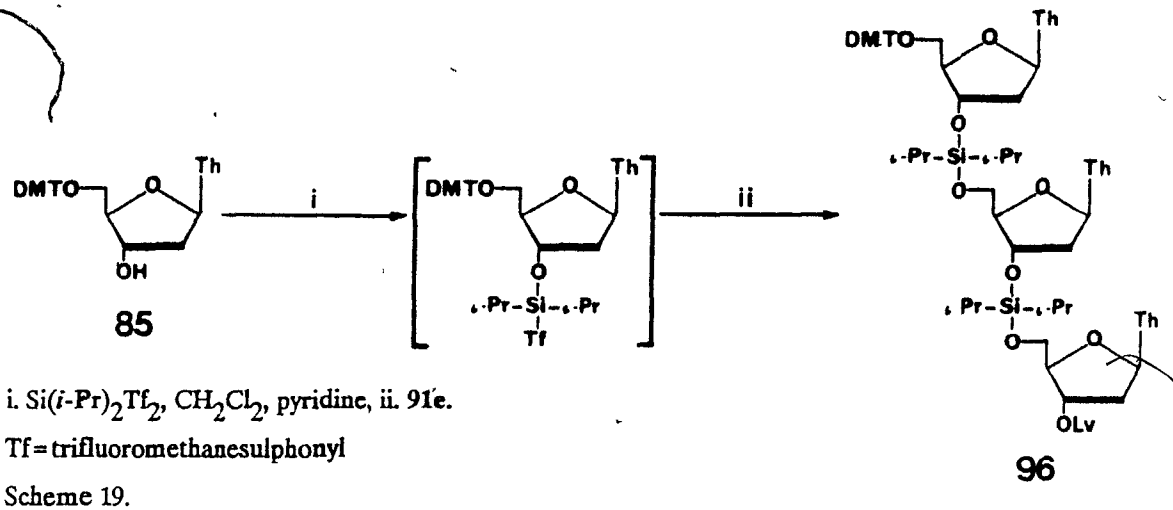
95

We attempted to synthesise dimer **88e** using our usual reaction conditions and the silyl triflate **95** (bis(trifluoromethanesulphonyl)diisopropylsilane). None of the desired product was found. We were somewhat baffled by this result, since we had expected that the triflate would be highly reactive, and that substitution by hydroxyl would be rapid. TLC showed mainly starting material after the usual workup. It was pointed out by a member of this laboratory (Dr. J. P. Roduit) that very active silylating agents may act to cleave ethers[146], such as the THF which we had used as the solvent. Switching to methylene chloride as the solvent gave the desired reaction. We were able to isolate the desired 3',5'-linked dimer **88e** in 47% yield. We later altered the ratio of reagents from 1:1.1·1 (**85**:silylating agent **87**) to 1. 8· 5, increasing the yield to 74%. The molecule was characterised by UV and ^{1}H and ^{29}Si NMR spectroscopy and low resolution fast atom bombardment (FAB) mass spectrometry. The UV spectrum is characteristic of an unmodified thymidine nucleoside. The most notable NMR characteristics are the presence of a single resonance at -7.5 in the ^{29}Si NMR (using the INEPT pulse sequence), and ^{1}H NMR signals corresponding to the methyl and methylene groups of the levulinyl protecting group, the methoxy groups of the dimethoxytrityl moiety, two incompletely resolved anomeric proton resonances, and the expected signal from the isopropyl groups attached to silicon.

With the desired dinucleotide analogue in hand, we then carried out the stepwise deprotection of this molecule (Scheme 17). Detritylation with zinc bromide as above gave us the partially protected dimer **91e** in good (75%) yield. Characterisation again involved the use of UV and ^{1}H NMR spectroscopy, both of which showed the expected features. The ^{1}H NMR is especially informative, showing the presence of the anomeric, and silyl protons, as well as the levulinyl group. This molecule could then be used in place of **87** (Scheme 19) in order to synthesise a trimer. At

146. M. E. Jung, M. A. Lyster, *J. Org. Chem.*, **42**, 3761 (1977).

first, however, we carried out the deprotection of the 3'-end to arrive at the fully deprotected material. Delevulination with hydrazine gave us the dimer **92e** in 64% yield after purification, the product being identified by ^{1}H NMR and UV spectroscopy. It is worth noting that no degradation of the silyl link was noted in this reaction even after several hours, pointing up the stability of the diisopropylsilyl group as compared to the diphenylsilyl analogue. The complete deprotection of the phenyl-*t*-butylsilyl analogue **88c** (the major diastereoisomer only) was also carried out *via* this same series of reactions, in somewhat lower yield (47% for the first step, 40% for the second).

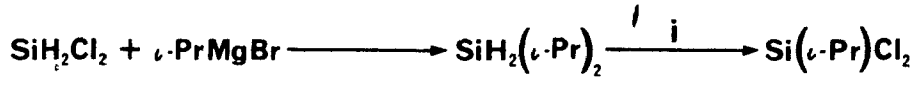


i. $Si(i\text{-}Pr)_2Tf_2$, CH_2Cl_2, pyridine, ii. **91e**.
Tf = trifluoromethanesulphonyl
Scheme 19.

Synthesis of Diisopropyldichlorosilane

At this point, we decided that the greater stability of the diisopropylsilyl link would be an advantage, since it allowed us to consider more vigorous deprotection conditions, if these became necessary. In particular, we wished to extend the series of analogues to include deoxyadenosine silylnucleotides. This most probably would require the use of some sort of protection, likely an amide, for the exocyclic amine. We had no confidence in the ability of the diphenylsilyl link to stand up to the deprotection conditions called for in that case. We decided to synthesise the commercially unavailable diisopropyldichlorosilane (Scheme 20) and compare it as a silylating agent to the silyl triflate (**95**) which we had been using. We were able to do this by first making diisopropylsilane after the procedure of West and Rochow[147].

147. R. West, E. G. Rochow, *J. Org. Chem.*, 18, 303 (1953).



i. $Pd(II)Cl_2$, CCl_4, 140°.

Scheme 20.

The ether solution of the Grignard reagent prepared from isopropyl bromide was placed in a three-necked flask Dichlorosilane was condensed into a graduated cylinder, then allowed to slowly evaporate through an inlet tube into the flask The flask was also fitted with a Dry Ice condenser to prevent escape of the silane (b. p 8°C). Aqueous workup and evaporation of the solvent followed by distillation gave the desired diisopropylsilane in moderate yield (46%) The required dichlorosilane was produced by the palladium catalysed reduction of carbon tetrachloride in a steel bomb[148] This is a very convenient reaction, as the byproducts may be removed by filtration of the catalyst and simple evaporation of the chloromethane products No attempt was made to identify the chlorocarbon byproducts, although Nagai *et al* reported the presence of both chloroform and methylene chloride in the product mixture, and accounted for the mass balance of the reaction by postulating the production of methyl chloride and methane Removal of the solvent followed by vacuum distillation gave the desired diisopropyldichlorosilane in 41% yield, or 19% for the two steps. This could no doubt be improved upon, but was sufficient for our purposes. The second reaction is highly exothermic, with the reaction vessel becoming very warm and the carbon tetrachloride boiling as soon as the catalyst (palladium (II) chloride) is added.

We attempted to synthesise **88e** using this new reagent. Our usual THF/pyridine conditions gave only very slow reaction, with completion not being reached after 48 h. The use of DMF/imidazole or THF/silver nitrate/pyridine, even at -78°C gave very poor results, the TLC showing mainly **89e** and **90e** as the reaction products. We were able to use this silylating agent later, as will be described below, for the deoxyadenosine analogue case.

Synthesis of Longer Thymidine Nucleotide Analogues

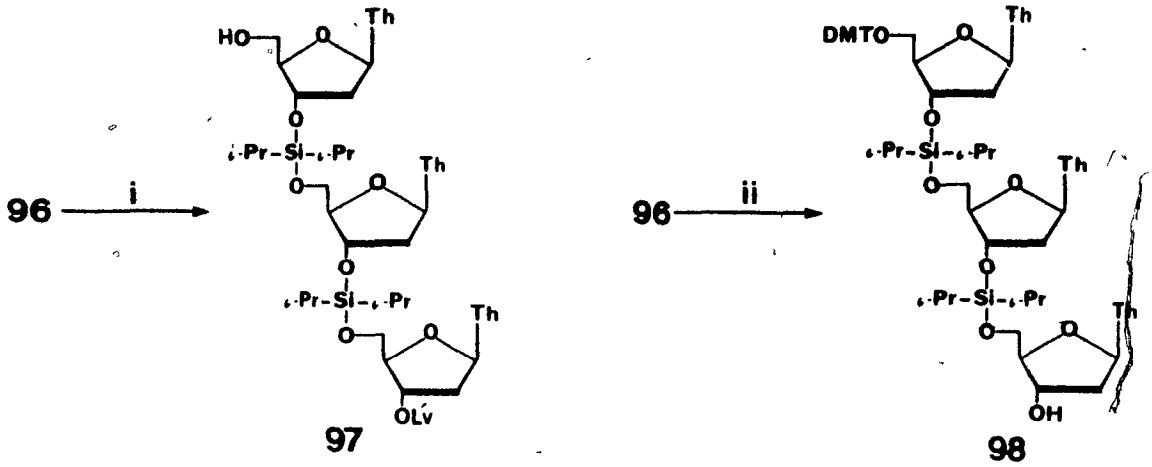
We then went on to synthesise the trinucleotide analogue **96** (Scheme 19). We used the diisopropylsilyl link, with the silyl triflate as the silylating agent. Under our standard conditions, we first formed the 5'-dimethoxytritylthymidine-3'-diisopropylsilyl triflate, then reacted it *in situ* with the partially deprotected dimer **91e**. The course of this reaction as shown by TLC was the same as seen

148. Y. Nagai, H. Matsumoto, T. Yagihara, K. Morishita, *Kogyo Kagaku Zasshi*, **71**, 1112 (1968).

during the synthesis of **88e**. The usual workup and silica gel column gave us the desired trimer **96** in reasonable (55%) yield. Changing the reagent ratio **85**:silylating agent:**91** from 1:1.1:1 to 2:1.6:1.3 increased the yield to 68%. **96** was identified by its ^{1}H NMR characteristics, with the main features being the presence of the resonances for the methyl group of the levulinyl moiety, the distinct signal due to the methoxy groups of the dimethoxytrityl group, and the three anomeric signals, two of which were overlapping, and one nearly completely resolved from them. In addition, the appropriate signals due to the silyl group were seen. ^{29}Si NMR shows two closely separated resonances, at -7.6 and -7 8 ppm, corresponding to the two silyl groups. The UV and low resolution FAB mass spectra also support the assigned structure.

We also wished to synthesise a longer oligomer in this series .We felt that a hexamer would be a reasonable target, since this is a large enough molecule to exhibit some of the well-known stacking and binding characteristics of natural nucleotides, but still short enough to be manageable in a solution phase synthesis. To this end, we decided to attempt a block condensation using two appropriately protected trimers. This would eliminate two coupling steps which would be required for a similar stepwise synthesis. Accordingly, we detritylated (Scheme 21a) a sample of the fully protected trimer, using trichloroacetic acid. This reaction is more rapid and convenient than the zinc bromide detritylation, and can be used here because of the greater stability of the diisopropylsilyl link. The desired partially protected molecule **97** was isolated in 73% yield after column chromatography

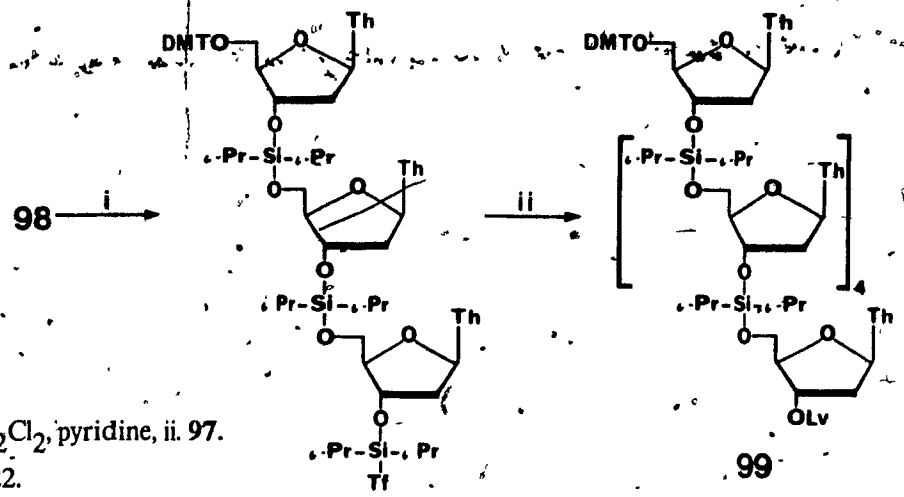
The other building block was prepared (Scheme 21b) by delevulination of a sample of the fully protected trimer with hydrazine, as described earlier. The reaction was allowed to proceed for ten minutes, with no indication of degradation as monitored by TLC. The 5'-protected trimer **98** was isolated in 88% yield after chromatography and precipitation from hexanes Both partially protected trimers were characterised by ^{1}H NMR and UV spectroscopy.



i. TCA/CH_2Cl_2, ii. Hydrazine (pyridine:acetic acd, 3:2)

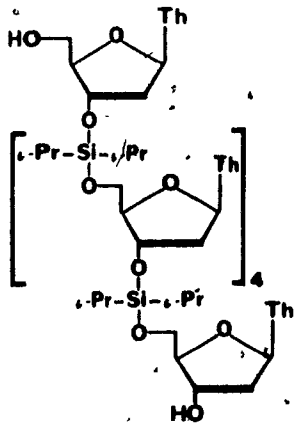
Scheme 21.

We then carried out the block coupling of these two partially deprotected molecules to arrive at the hexanucleotide analogue 99 (Scheme 22). This reaction was done on a particularly small scale (.17 mmol) due to the limited amount of the precursors which was available to us. We again used the triflate as the silylating agent, and a much longer reaction time (1.5 h for the first reaction, 16 h for the second) was allowed to ensure complete reaction at both steps. The reaction went quite smoothly, with TLC showing complete reaction. The usual workup gave a three-component mixture (TLC) as expected. In this case, the three products (two tritylated) were very closely separated. Chromatographic separation was very difficult, requiring one column, and two preparative plate purifications. This no doubt contributed to the poor yield of the desired product 99 (12%). The initial TLC of the reaction showed that the actual yield was probably closer to 50%. However, removal of the symmetrically linked byproducts proved much more difficult than anticipated. The desired all-3',5'-linked hexathymidylic acid analogue 99 was identified by its ^1H NMR spectrum, which showed the presence of the appropriate protecting groups, and the correct ratio of anomeric to isopropyl protons. UV spectral analysis indicated that no base modification had occurred.



i. 95, CH_2Cl_2, pyridine, ii. 97.
Scheme 22.

Deprotection was carried out in the same order as above. The dimethoxytrityl group at the 5'-end of the molecule was removed using TCA in methylene chloride. After workup using aqueous sodium bicarbonate, the organic layer was evaporated to yield a foam. TLC indicated the presence of a relatively slow-moving material, and a very fast spot which was assigned to the dimethoxytritanol byproduct of this reaction. No tritylated nucleotide analogue was detected by TLC. In order to preserve the compound, the crude material was not purified but immediately delevulinated using ammonium hydroxide and methanol, with dioxane added to completely dissolve the material. After stirring overnight (16 h) at room temperature, the solvents were removed by evaporation and the product purified on a silica gel column to yield the final product (100, 14 mg).



100

Again, this material was identified by its ^1H NMR spectrum, showing the appropriate ratio of anomeric to isopropyl signals. The UV spectrum supported the assigned structure.

The deprotection yield was acceptable, but the difficulty in purifying the fully protected material points up one of the primary weaknesses in preparing such molecules by solution phase synthesis. Even though the actual coupling reaction may be reasonably efficient, purification may be sufficiently difficult to seriously hamper the overall efficiency of the synthesis. Solid phase synthesis offers an advantage here, since it allows for highly selective couplings, partial deprotection on the solid support, and purification only at the last step, instead of at each intermediate coupling. Certainly, for longer sequences, solid phase synthesis would have to be given serious consideration.

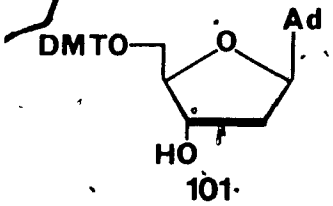
Deoxyadenosine Nucleotide Analogues

As mentioned in the introduction to this chapter, we wished to make silyl nucleotide analogues of a representative purine nucleoside. To this end, we chose 2'-deoxyadenosine as that substrate. This presents us with the common protecting group problems of nucleotide chemistry, and allows us to attempt the synthesis of a hexamer which is complementary to the thymidine oligomer described above. Chronologically, this work was undertaken after the thymidine work was well underway. Thus, we had a good idea of the best silylating conditions and agents to use. We were faced with an extra protecting group problem. Deoxyadenosine contains an exocyclic amino group at the 6-position of the base. It was possible that such a functionality might be sufficiently reactive to become involved in the silylation reaction. We had to decide whether or not to protect this position. If it was protected, deprotection conditions would have to be such that the silyl link would be untouched. Normally, the amino group is protected as an amide, usually with benzoyl. The deprotection of this position is usually done with ammonium hydroxide and methanol, at ambient or elevated temperatures. Clearly, as described above, the diphenylsilyl link would not withstand these conditions. Thus, if we wished to use this link, the amino group would have to be unprotected. Alternatively, we could use a more stable silyl link, and protect the amino group. At that time, we had not yet explored the stability of the diisopropylsilyl link.

Synthesis and Stability of Dexoyadenosine Analogues.

Initially, we wished to synthesise the deoxyadenosine analogue of compound **88** using the diphenylsilyl link. Accordingly, we reacted 5'-dimethoxytrityl-2'-deoxyadenosine (**101**) with diphenyldichlorosilane under the same conditions used for the thymidine case (THF-pyridine, -78°C, Scheme 24). The reaction was allowed to proceed for a time, then the second nucleoside,

3'-levulinyl-2'-deoxyadenosine (**102**, prepared as for **87**) was added. After an appropriate time, as judged by TLC, the reaction was worked up.



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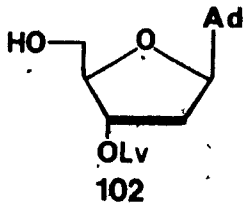

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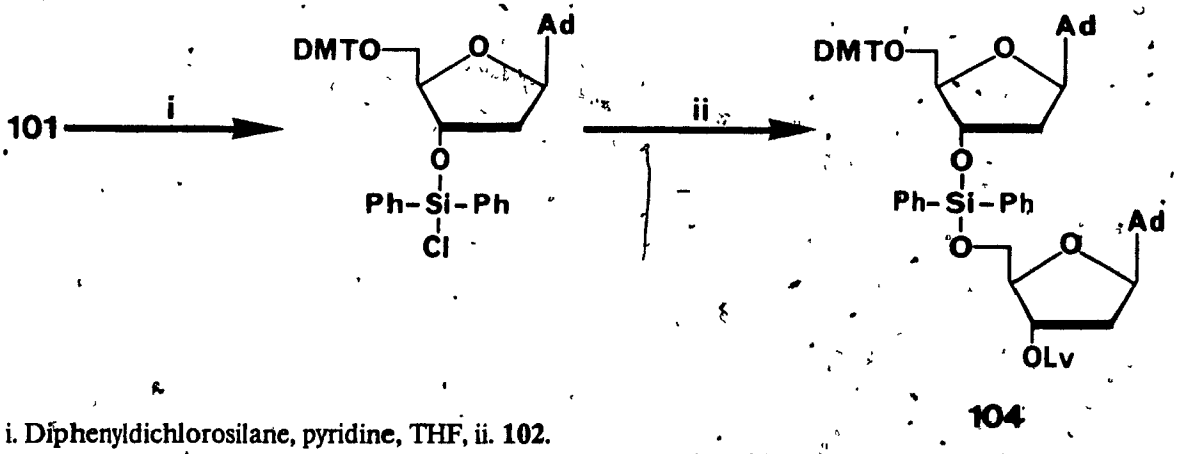


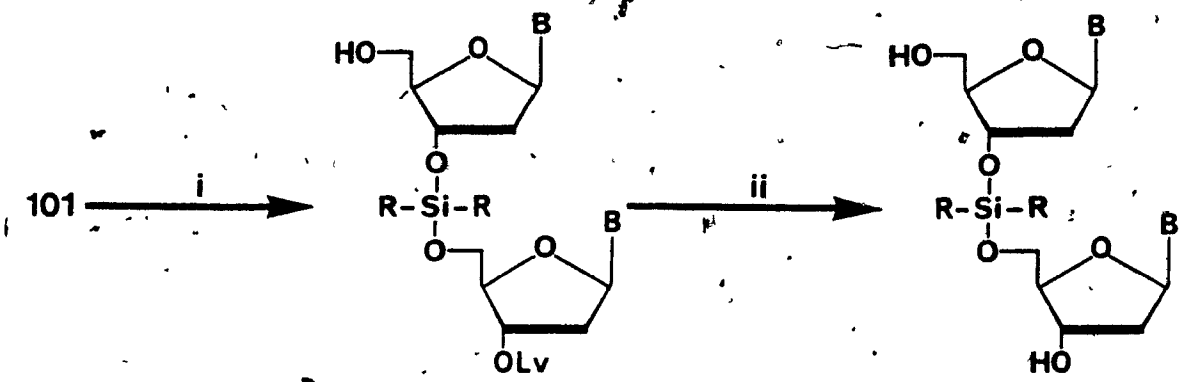
104

i. Diphenyldichlorosilane, pyridine, THF, ii. **102.**

Scheme 23.

TLC showed that this reaction proceeded in the same way as the thymidine reaction. Two tritylated products appeared after the first nucleoside was added, the faster of which was consumed after the second nucleoside was allowed to react. This disappearance coincided with the appearance of a new, slower-moving tritylated material. A very slow, nontritylated material was also observed in the final product mixture. Column chromatography gave the desired 3',5'-linked dimer 104 in about 45% yield.

Deprotection of this molecule (Scheme 24) was accomplished as before. Zinc bromide detritylation gave the partially protected dimer **105a** in good (86%) yield. Delevulination with hydrazine, allowed to react for three minutes, led to the completely deprotected molecule **106a** in 62% yield as the only nucleosidic product.

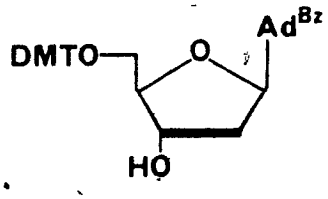


105 106

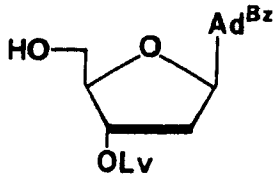
R, B = a, phenyl, adenine; b, *i*-Pr, N^6-benzoyladenine; c, *i*-Pr, adenine.
i. TCA/CH_2Cl_2, ii. Hydrazine (pyridine:acetic acid, 3:2) for a and b, NH_4OH:MeOH for c.

Scheme 24.

At this time, we were having a degree of success with the diisopropylsilyl link in the thymidine series. We had found, as described above, that it was significantly more stable than the diphenylsilyl link. Thus, we applied this idea to the deoxyadenosine series. Using 5'-dimethoxytrityl-2'-deoxyadenosine 101 as the first nucleoside, we attempted to make the fully protected dimer 104c in the same way as for the thymidine dimer 88e, with the silyl triflate as the silylating agent. We found, however, that no predominant product resulted. TLC analysis of the reaction showed a complex mixture of at least four major products and several minor ones. We therefore turned to diisopropyldichlorosilane, described above, as the silylating agent. We also decided at this point that it would be better safe than sorry as far as the amino protection was concerned, so we synthesised N^6-benzoyl-5'-dimethoxytrityl-2'-deoxyadenosine 107 by a literature procedure and from this, synthesised the appropriate 3'-levulinated benzoylated deoxyadenosine 108 by the same procedure as before, namely, levulination of the tritylated nucleoside, followed by detritylation.



107

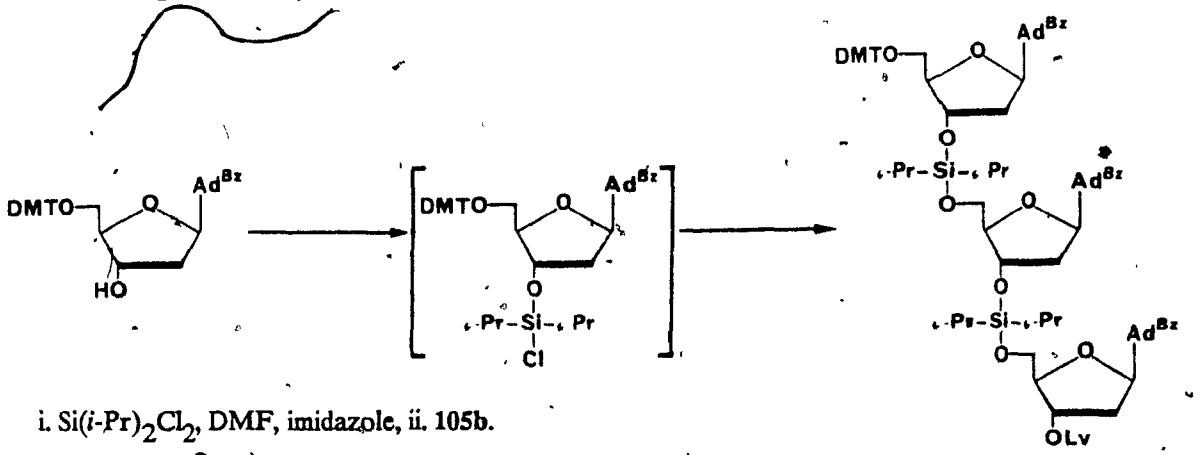


108

Reaction of the diisopropyldichlorosilane with 107 and 108 by the usual reaction sequence (analogous to Scheme 23), in anhydrous DMF, using imidazole as the base, led to isolation of the desired 3',5'-linked dimer **104b** in 47% yield. Changing the ratio 107:silylating agent:108 from 1:1.1:1 to 1:.8:.5 improved the yield of **104b** to 60.5%. As usual, the dimer was identified from its ^{1}H NMR spectrum, which showed signals for the levulinyl, dimethoxytrityl and diisopropylsilyl groups, as well as the two anomeric protons. The INEPT ^{29}Si NMR showed a single resonance (at -7.1 ppm), as expected. Low resolution FAB mass spectrometry and UV spectroscopy agreed with the assigned structure.

Detritylation of this material to give the partially protected dimer **105b** (Scheme 24) was accomplished with 3% TCA in methylene chloride, with the product isolated in 67% yield. It is worth noting here that N^6-protected adenosines, and 2'-deoxyadenosines in particular, are highly susceptible to acid-promoted depurination, as mentioned in the preceding chapter. Thus it is necessary to keep the reaction time to a minimum. For this reason, we avoided the use of acetic acid or zinc bromide to carry out the detritylation. With the appropriate care, however, this reaction presents no problems. ^{1}H NMR showed the appropriate signals, and the UV spectrum was characteristic of a benzoylated adenine nucleoside.

We exposed some of this material to the standard hydrazine delevulination conditions for the purpose of determining its stability. After a few minutes, all the starting material had been consumed, apparently converted to a slightly more polar material, which was most probably the delevulinated compound **106b**. This product was stable to these conditions for at least 28 h, underlining the stability of this silyl link.



i. Si(*i*-Pr)$_2$Cl$_2$, DMF, imidazole, ii. **105b**.

Scheme 25. **109**

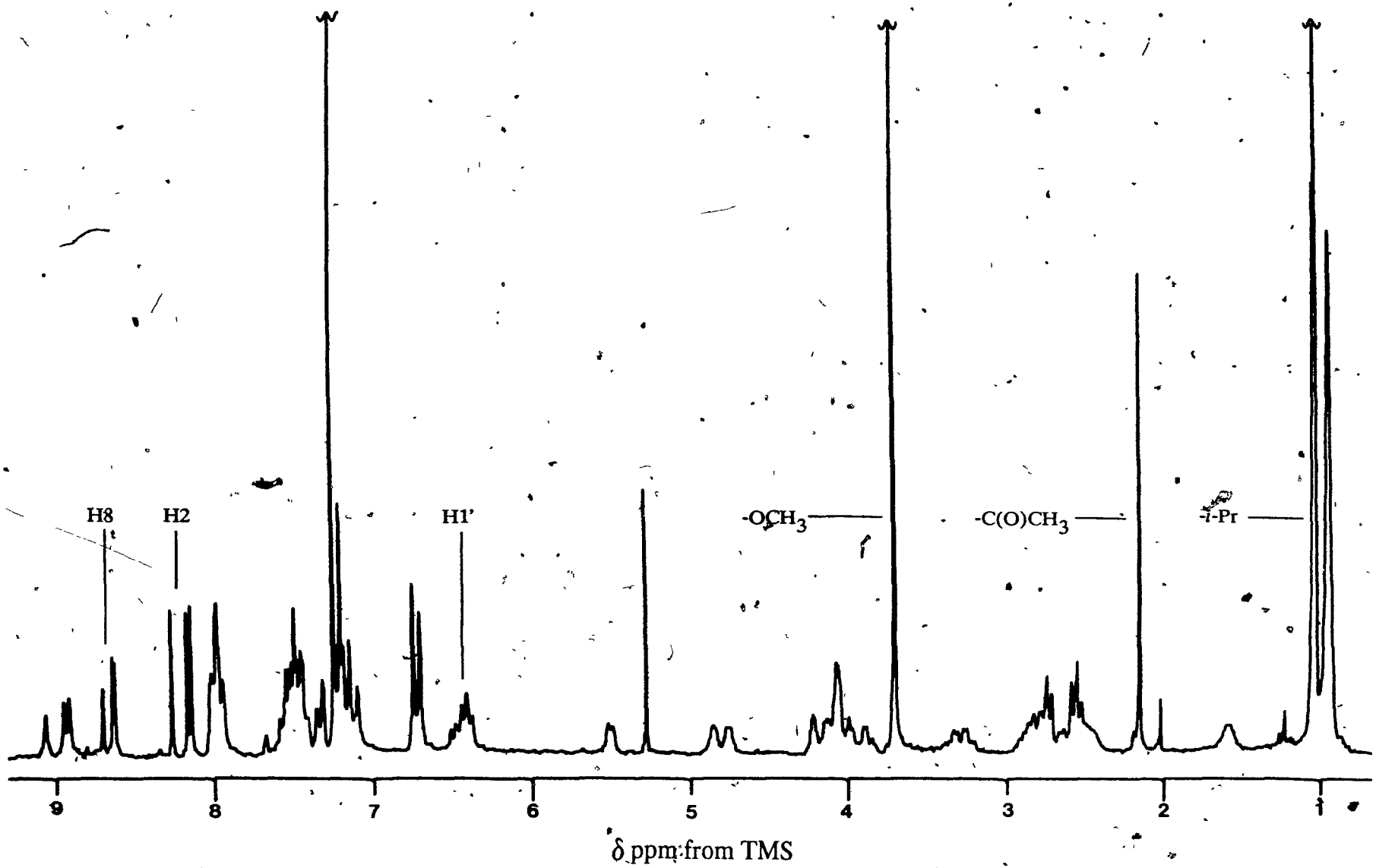


Fig. 11. ^{1}H NMR of **105b**.

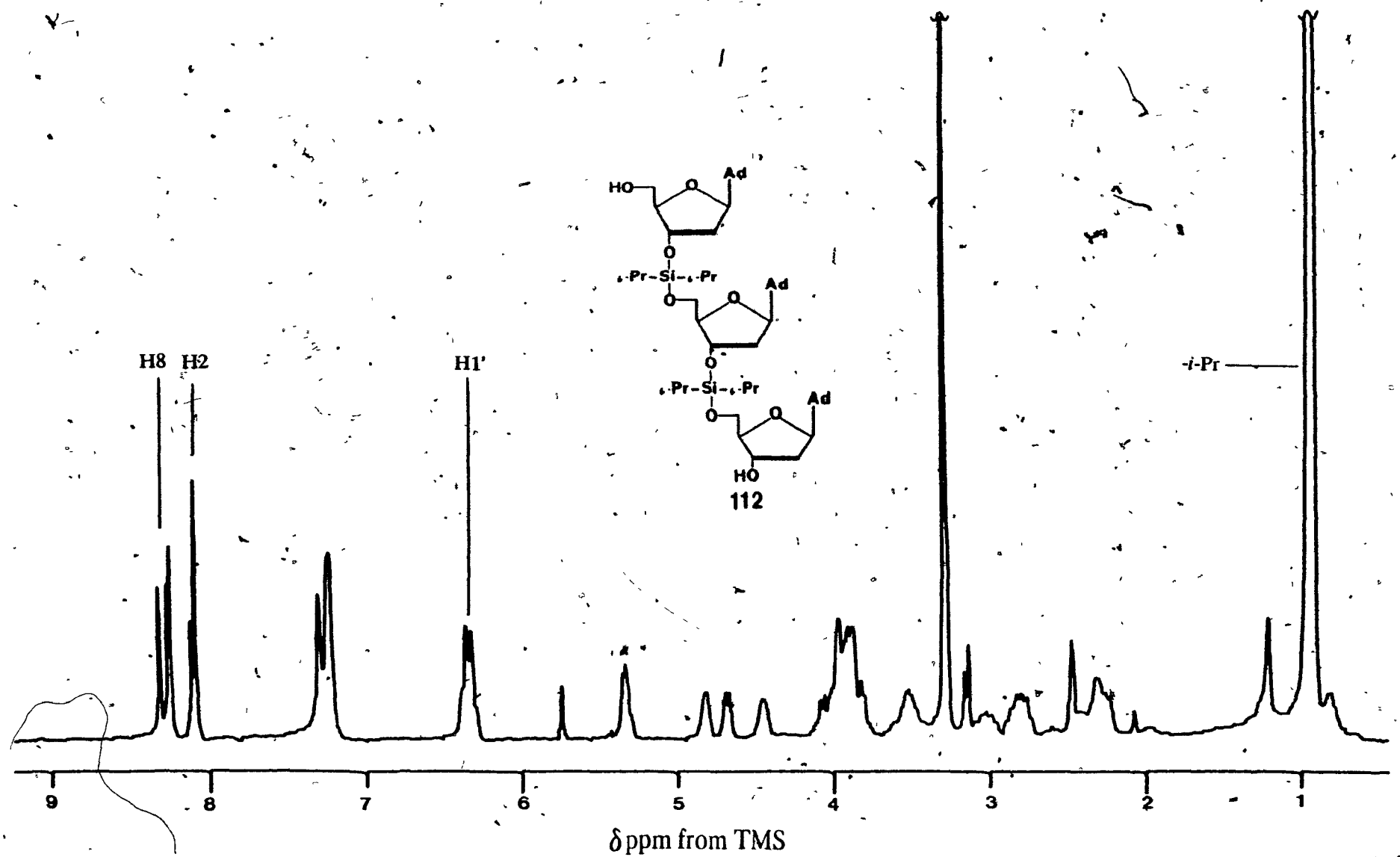
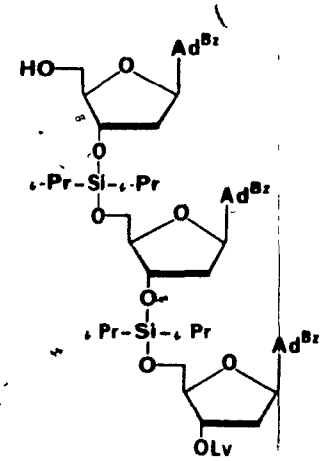
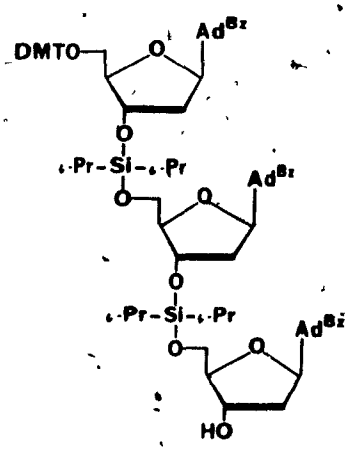


Fig. 12. ^{1}H NMR of 112.

We then proceeded to make the trimer of this series (**109**, Scheme 25). Tritylated nucleoside **107** was coupled with detritylated dimer **105b** under the same conditions as outlined above. The usual workup and silica gel column purification yielded the desired 3',5'-linked trimer **109** in 45% yield. The product was identified by ^{1}H and (INEPT) ^{29}Si NMR and UV spectral analysis, as well as low resolution FAB mass spectrometry. The ^{1}H NMR resonances (Fig 11) were difficult to completely assign, due to the extensive overlap of the three nucleoside spin systems However, the anomeric protons stand clear, with two overlapping completely, and one nearly resolved In addition, one can easily distinguish the sharp signal at 3 72 ppm due to the methoxy protons of the 5'-protecting group, and the singlet corresponding to methyl moiety of the levulinyl group at 2 17 ppm The methylene protons of this protecting group (2 5 and 2 7 ppm) give signals which obscure those due to the 2'-protons of the nucleosides The signals due to the isopropyl groups attached to silicon appear at about 1 ppm downfield from TMS, as expected, and the ratio of signal integrations is correct. We also observe three sets of base protons (H2, *ca* 8 1 ppm, and H8, *ca* 8 5 ppm) due to the hydrogens at C-2 and C-8 of the heterocyclic adenine bases. The ^{29}Si NMR shows two signals at -7.2 and -7 7 ppm. The UV spectrum exhibits the expected absorption maximum at 270 nm, indicating that the benzoyl protecting groups remain intact.

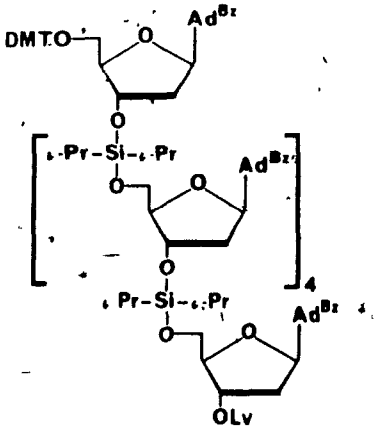


110

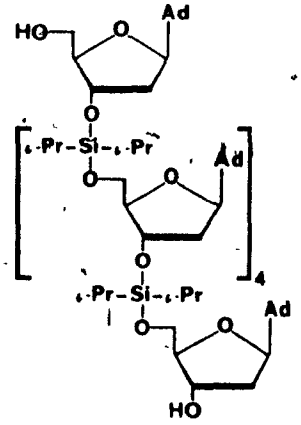


111

At this point, we prepared the partially deprotected trimers **110** and **111**, in order to proceed with a block coupling as described earlier for the thymidine series. Compound **110** was prepared by trichloroacetic acid detritylation, keeping the reaction time as short as possible (*vide supra*). The reaction proceeded quite cleanly, the product being isolated as a white foam in 67% yield after column chromatography The delevulinated trimer **111** was prepared by reaction of **110** with hydrazine in pyridine-acetic acid, 3 2, as usual, and isolated, again as a white foam, in 81% yield. Both products were identified by their ^{1}H NMR and UV spectra. A small amount of **110** was completely deprotected with ammonium hydroxide:methanol dioxane to give **112**, and its ^{1}H spectrum is shown (Fig 12).



113



114

With the required trimer blocks in hand, we proceeded with the synthesis of the hexanucleotide analogue **113**. The reaction was carried out in the same manner as for the thymidine hexanucleotide analogue (*vide supra*) We allowed a somewhat longer reaction time than usual at both steps to ensure complete reaction, in view of the fairly laborious procedure for the preparation of the precursors The desired product was isolated after two columns, one to remove the nontritylated product, a second to separate the two tritylated materials

The hexamer was isolated in 11 5% yield, and identified as such from its proton NMR spectral data, with supporting evidence from the UV spectrum Although the NMR spectrum was, not surprisingly, quite complicated, several essential features could be distinguished. First, the resonances for both hydroxyl protecting groups were clearly visible. Second, the ratio of the integration of the anomeric signals to that of the isopropyl signals was as expected. Third, the correct number and intensity of base protons (C-2 and C-8) were observed. Interestingly, the chromatographic mobility of the products from this experiment differed from that found in all our previous syntheses. In those cases, the product resulting from 5',5'-coupling was the slowest-moving, with the other symmetrical product moving the fastest, and the desired 3',5'-linked

material having an intermediate mobility. In this case, the desired product was the slowest moving, and the nontritylated, 5',5'-linked product the fastest NMR analysis, however, easily distinguished the two tritylated materials, by showing the presence of the levulinyl protecting group in the desired hexamer , and this group's absence in the symmetrically linked hexamer byproduct.

In deprotecting this molecule, we encountered a serious problem In all previous cases, in both the thymidine and deoxyadenosine series, we had carried out the detritylation step first, followed by removal of benzoyl and levulinyl groups as appropriate (*vide supra*). In the present case, detritylation as usual with trichloroacetic acid led to the production of a single nontritylated, UV-absorbing product. TLC and UV analysis indicated that this material was the product of depurination. The ^{1}H NMR of this material showed there were no signals assignable to sugar or silyl protons. Only the base protons showed resonances. Comparison with the NMR spectrum of N^6-benzoyl adenine provided by M. Damha of this laboratory, showed that the product of the detritylation was indeed the protected base, and that complete depurination had occurred. As mentioned above, N^6-protected deoxyadenosines are especially susceptible to this reaction, but our previous experience had led us to believe that our detritylation conditions were sufficiently mild to be safe. It was necessary to repeat the synthesis of the hexanucleotide analogue. In this case, we carried out the delevulination and debenzoylation in one step, using methanol·ammonium hydroxide:dioxane. Removal of the base protection enhances the acid-stability of deoxyadenosines. We then carried out the detritylation successfully with trichloroacetic acid in methylene chloride. The final deprotected hexamer **114** was isolated in 35% yield, after silica gel column chromatography and identified by its ^{1}H NMR spectrum, with the UV spectrum showing the appropriate unmodified adenine nucleoside absorption.

Physical Studies on the Hexamers. CD and Hypochromicity

Given their central role in the function of biological systems, it is not surprising that the physical properties of nucleotides have been extensively studied. One of the most important branches of this area has been that of the study of the optical properties of nucleotides, both in the monomer form, and in oligomers and polymers. Nucleosides and nucleotides, by virtue of their heterocyclic bases, display UV absorption spectra. Due to the chirality of their sugar moieties, nucleosides and nucleotides are also optically active. Both the UV absorption and the optical activity are sensitive to interactions within and between nucleotides. This circumstance has been widely exploited in the study of nucleic acids, and, in particular, in the study of the spatial relationships between the consitituent parts of nucleic acids.

Interactions between the chromophores may take place within a single strand of ribonucleic or deoxyribonucleic acid. The heterocyclic base, which is a flat, aromatic structure, may "stack" with the base of the adjacent nucleotide. This stacking interaction is responsible for the stabilisation of the strand in a specific orientation. It also has an effect on the optical properties of the molecule. The observed effect is to lower the UV absorption of the whole molecule, relative to its component mononucleotides. Thus, a measurement of the optical density of a given solution of an oligonucleotide, for example, followed by the degradation of that material to the mononucleotide stage and measurement of the optical density of the degraded material, usually shows an increased optical absorption. The difference between the final and initial measurements, is referred to as the degree of hypochromicity of the original material, and is usually expressed in terms of a percentage of the absorption of the mononucleotide constituents. For example, if the absorption of the intact oligonucleotide is 90% that of its mononucleotide components, a hypochromicity of 10% is said to exist. The degradation is usually carried out enzymatically, although chemical degradation is also used. The change in UV absorption with increasing temperature may also be used. This, however, depends upon the assumption that the stacking interaction is completely eliminated at a sufficiently high temperature.

It is well known that when double-stranded DNA is heated, its UV absorption increases in intensity. When absorption is plotted against temperature, a distinct S-shaped curve is observed. This is due to the separation of the two strands from one another. Thus, the UV absorption of the duplex is less than that of the separate strands from which it is formed. The temperature range over which the largest part of this transition takes place is often very narrow, frequently only a few degrees. The center point of the transition (the point at which the concavity of the temperature *versus* absorption curve changes) is called the melting temperature, T_m. Because the melting temperature is dependent upon the strength of the binding between the complementary strands, such data may be used to determine thermodynamic parameters. The strength of binding is in large part due to the nucleotide composition of the two strands, so that an approximation of the ratio of G:C to A:T base pairs may be obtained. G:C base pairing is significantly stronger than A:T pairing.

Since we wished to compare our nucleotide analogues to their natural counterparts, we examined them for evidence of this hypochromicity, and also for duplex formation with natural nucleotides and with one another. We studied the hypochromicity by measuring the UV absorbance of a sample of the nucleotide analogue, then degrading the sample with concentrated ammonium hydroxide:methanol at 60°C overnight. These conditions are sufficiently vigorous to degrade the diisopropylsilyl link completely. The sample was lyopholised, then the residue was dissolved in the same volume of water as before. The absorbance was then measured again, on the same instrument

and in the same UV cell. We found that there was indeed an increase in absorbance on the latter measurement. In the case of the thymidine hexamer, this amounted to 9.7% (as compared to 10.4 % for the natural hexanucleotide)[133], while the deoxyadenosine hexamer displayed no hypochromicity, within the limits of this measurement. The error in this measurement is probably fairly high, with the volume measurement probably being accurate to no better than 1% at each step. Both values given here are the result of two measurements. The results indicated to us that the thymidine hexanucleotide analogue was able to assume a conformation in solution which allowed base:base interactions, as in the case of the natural nucleotides. The lack of observed hypochromicity in the 2'-deoxyadenosine hexanucleotide analogue led us to believe that the base:base interactions were probably of low magnitude.

We also wished to examine the interaction between complementary strands of our analogues, and between the analogues and their natural counterparts. Unfortunately, we were not able to dissolve our analogues in water in sufficiently high concentrations to achieve binding. The maximum absorbance we were able to obtain with the thymidine hexanucleotide analogue in pure water was *ca.* 3.4, which corresponds to a concentration of .06 mM. For the 2'-deoxyadenosine hexanucleotide, we could only get solutions of .71 absorbance, a concentration of about .01 mM. In retrospect, this aspect of the work would have been better served had we synthesised oligomers of deoxyguanosine and deoxycytidine. Thymidine and 2'-deoxyadenosine were chosen as the subjects of this study due to the simplicity of their handling, presenting minimal protecting group problems for a pyrimidine and a purine, respectively. Each is also the least expensive of its class. But base pairing between 2'-deoxycytidine and 2'-deoxyguanosine sequences is substantially stronger than between thymidine and 2'-deoxyadenosine sequences.

As far as the solubility problem is concerned, the neutral character of the internucleotide link is clearly not the only factor which comes into play. Many other uncharged nucleotide analogues have been successfully studied for their binding properties. In particular, the methylphosphonates (see General Introduction) have shown quite good water solubility.

The interaction of two or more adjacent bases also may give a clue as to their spatial arrangement. If the chromophores are related in some asymmetrical way, then they may display a circular dichroic (CD) spectrum. A very lucid description of the optical properties of nucleic acids has been given by Bush[149]. The following outline is largely derived from this source. If we consider a beam of plane polarised light as being composed of two circularly polarised components (E_l and E_r), it is entirely possible that the indices of refraction for these two components in a given sample

149. C. A. Bush, *Basic Principles in Nucleic Acid Chemistry*, P. O. P. Ts'o, ed., Academic Press, New York, New York, 1974, p. 92.

may be different. The two components E_l and E_r may be envisaged as describing two helices of opposite handedness. If we consider the two enantiomers of an optically active compound as also being opposite-handed helices, then the fact that the two components have different interactions with a given enantiomer is not surprising. The emerging beam would then display a rotation of the plane of polarisation. This is optical rotation, a very common and well-studied phenomenon in chemistry. The indices of refraction are different, and optical activity is observed. If the indices of refraction for E_l and E_r are different, then we may also expect to find that their extinction coefficients may also be different. This is, in fact, the case. The difference in extinction coefficients between the two circularly polarised components is called circular dichroism. It is generally reported in terms of molar ellipticity [θ]. The origin of this term is in the fact that the emerging light is no longer plane polarised, but rather elliptically polarised due to the unequal absorption of the two circular components. In practice, the sample is exposed alternately to right- and left-handed circularly polarised light and the difference in absorptions measured. A CD band is often called a Cotton effect, after A. Cotton, who first described the phenomenon. The CD of mono-, oligo- and polynucleotides has been extensively studied, and may be used to determine the existence and nature of various interactions along and between strands.

It is important to note that the chromophores (bases) of nucleotides are not in themselves optically active. The optical activity of nucleotides arises from the asymmetry of the sugar portion of the molecules. This places the chromophores in an asymmetrical environment. Thus even mononucleotides display weak CD activity. There is another effect due to the fact that adjacent bases in a nucleotide sequence may be held in close proximity. Two chromophores held close to one another will interact *via* their electric fields. This interaction gives rise to a splitting of their absorption bands. This is called exciton splitting. It is not readily apparent in the UV absorption of nucleic acids, but may be seen in their CD (and optical rotatory dispersion, ORD) spectra. In this case, the split bands give Cotton effects of opposite sign. Because the sign (that is positive first and negative second Cotton effect or *vice versa*) of the double Cotton effect depends upon the spatial orientation between the chromophores (specifically the alignment of the transition moment vectors), the CD spectrum may be used to

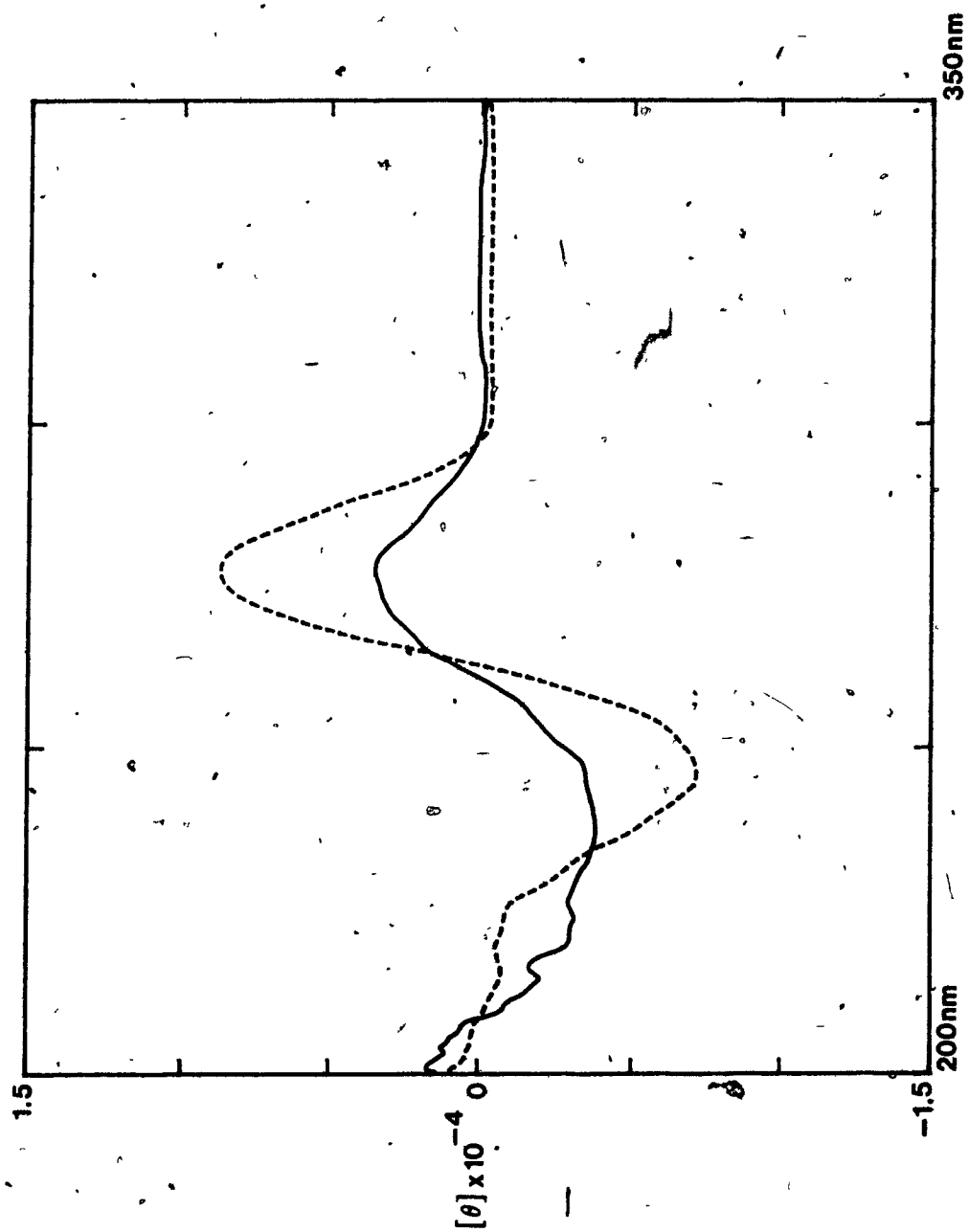


Fig. 13. CD spectra of **100** (solid) and d$T(pdT)_5$ (broken).

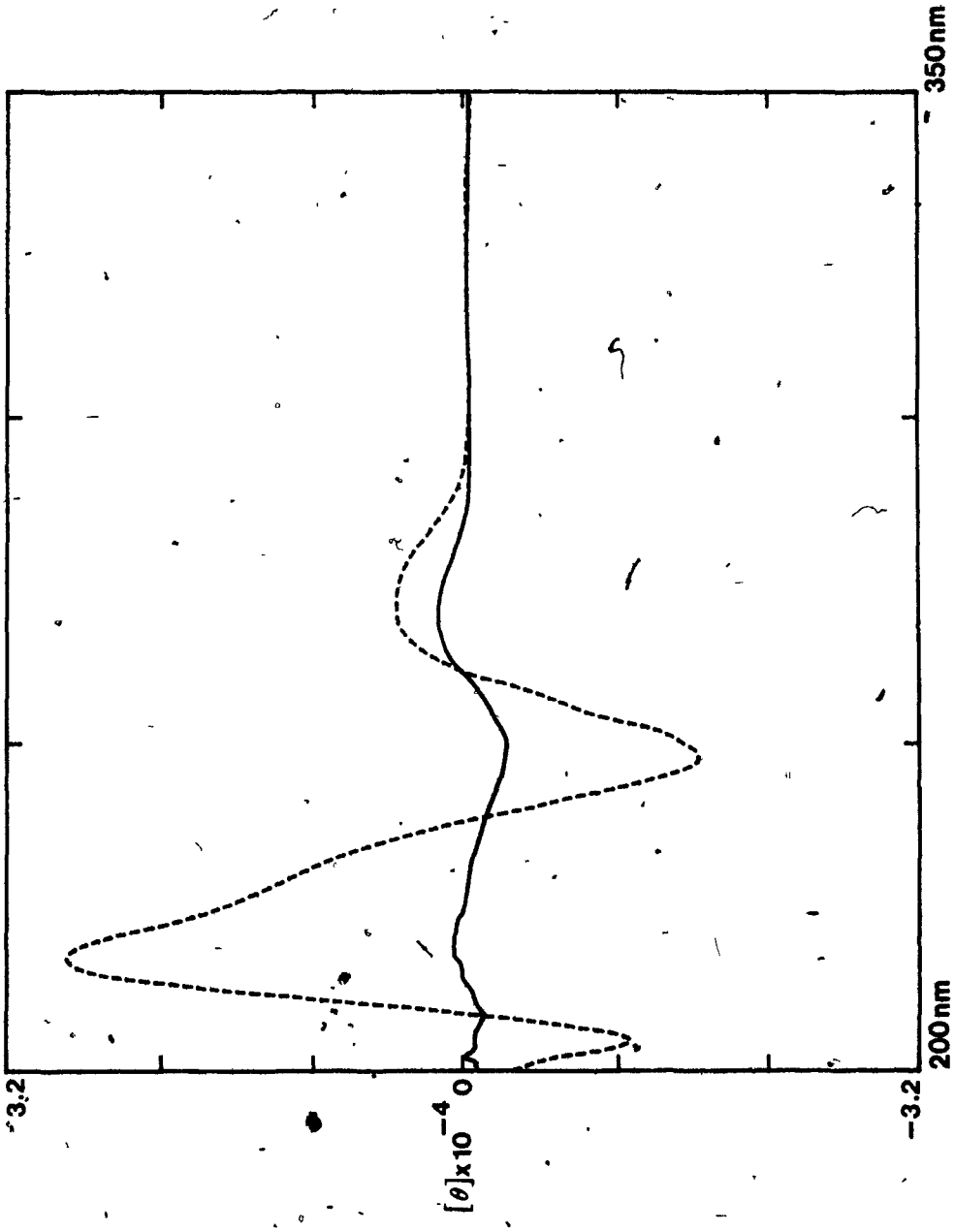


Fig. 14. CD spectra of 114 (solid) and $dA(pdA)_5$ (broken).

assign the sense of helicity of an oligonucleotide, if only one type of base is involved. If more than one type of base is present (that is, if the polynucleotide is not a homopolymer) then the interpretation is more difficult, but information about the helix may still be obtained. In such a case, however, it is often necessary to calculate the theoretical CD spectrum for the expected configuration, then compare it to the empirically determined spectrum.

In order to further compare our analogues to their natural counterparts, we examined the circular dichroism spectra. The CD spectra of nucleotides are quite sensitive to their secondary arrangement, and are frequently affected by temperature and ionic strength variations. Because of the solubility problems mentioned above, our CD studies were carried out in pure water, as opposed to buffer or salt solutions. We were gratified to see that our analogues exhibited a positive first Cotton effect, and a negative second Cotton effect. The thymidine hexanucleotide analogue **100** exhibited a molar ellipticity $[\theta]$ of 3.63×10^3 ^{o} cm^{-1} M^{-1} at 268 nm and -3.63×10^3 at 225 nm, as compared to 9.03×10^{-3} and -7.08×10^3 at the maximum and minimum for the natural hexanucleotide (Fig. 13). The CD spectrum of the natural hexathymidylic acid is also presented in the same figure. The 2'-deoxyadenosine analogue **114** has $[\theta] = 2.36 \times 10^3$ at 270 nm and $[\theta] = 2.40 \times 10^3$ at 250 nm. Again the natural hexaadenylic acid CD spectrum is shown for comparison. In this case, $[\theta] = 5.28 \times 10^3$ at 270 nm, and -1.68×10^4 at 250 nm (Fig. 14).

In homo-oligonucleotides, this CD spectral line shape is characteristic of a right-handed helix. The CD spectra of the two hexamers **100** and **114** were not appreciably changed on heating to 75^{o}C. This indicates that the orientation of the bases with respect to one another is not seriously affected by heating to this temperature. This complements the observation mentioned above that heating to a similar temperature does not affect the UV spectra of these two compounds. The intensity of the CD spectra of our analogues is lower than for the natural oligonucleotides, but of similar shape. This would indicate that the geometry of the silyl nucleotide analogues is similar to that of the natural nucleotides, but not identical.

Instrumental Techniques.

The characterisation of the products described in this chapter was carried out using several instrumental techniques. Foremost among these was proton NMR. This allowed us to distinguish between the various possible coupling products. We could easily determine which was the product of symmetrical 5',5' coupling simply by exposing the TLC of the reaction mixture to acid. The characteristic orange colour of the dimethoxytrityl cation showed which products were tritylated. Differentiating the tritylated products was more difficult. The purified materials could be examined by ^{1}H NMR. The anomeric proton (H1') signals of deoxynucleosides are

characteristically in the 6.0-6.8 ppm region, and are usually uncomplicated by other resonances. The ratio of anomeric protons to those of the silyl group showed whether there were one or two nucleosides present. In the case of dinucleotide analogues, the two anomeric signals were usually partly resolved, one from another. The resonances of the various protecting groups were also used diagnostically. The methoxy groups of the dimethoxytrityl moieties give rise to sharp singlets. Interestingly, although two methoxy groups are present, only one signal was observed in all cases. The methyl group of the levulinyl protection also gives rise to a sharp singlet. The methylene resonances of this protecting group partly obscure the signals due to H2' and H2". The presence or absence of the levulinyl protecting group, as well as the ratio of methoxy to anomeric protons differentiated the 3',5'-linked products from the 3',3'-linked materials. Two spectra, one of a fully protected dinucleotide analogue (**109**) and one of the same molecule after deprotection (**112**) have been presented as examples (Figs. 11 and 12).

The UV spectra of nucleosides and nucleotides are fairly sensitive to the extent of base modification. Thus, the similarity of the UV spectra of our products to those of the starting materials allowed us to be reasonably confident that no reaction had occurred at the heterocyclic bases. In particular, it was clear that the benzoyl group had remained at the N^6 position of the 2'-dexoyadenosine derivatives until we wished to remove it.

Some of the products were also examined by ^{29}Si NMR. Silicon NMR is difficult and inconvenient due to the low natural abundance of the observable ^{29}Si isotope (4.9%), its low and negative gyromagnetic ratio, and its typically long spin-lattice relaxation times. This inherent insensitivity means that silicon NMR by the normal pulse sequences requires large amounts of material and/or long acquisition times (for Fourier transform instruments). Fortunately, pulse sequences have been developed which improve this situation. We used the INEPT (Insensitive Nuclei Enhancement by Polarisation Transfer) pulse sequence[150] for the ^{29}Si NMR data reported in this thesis. This technique takes advantage of the high natural abundance of hydrogen. Magnetisation is transferred from the abundant, sensitive and rapidly relaxing hydrogen nucleus to the relatively rare and insensitive silicon nucleus of interest. This both enhances the desired signal and allows the acquisition of more transients in a given period of time.

150. G. A. Morris, R. Freeman, *J. Am. Chem. Soc.*, **101**, 760 (1979).

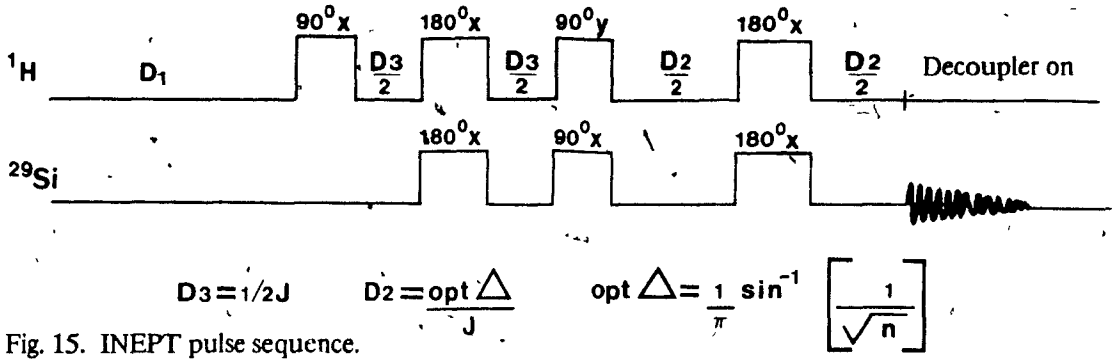


Fig. 15. INEPT pulse sequence.

In order to take advantage of this pulse sequence, it is necessary to determine the coupling constant $J_{Si,H}$ for the system under examination, and also to determine the number of hydrogen nuclei coupled to silicon. Once these values have been determined, they may be used to calculate the delay times D2 and D3 (Fig. 15, n=number of coupled hydrogens, J=Si-H coupling constant). The coupling constant $J_{Si,H}$ may in principle be determined from the coupled proton spectrum by measuring the satellite peaks of the silyl protons. This is not usually practical, however, due to the extremely low intensity of these satellites. In practice, one usually arrives at the correct values for D2 and D3 by assuming a value for the coupling constant (and thus D3) and varying the number of coupled protons used to calculate D2. Once the value of coupled hydrogens giving maximum enhancement has been determined, $J_{Si,H}$ is varied and D2 and D3 recalculated to give the best signal. In our case, we determined the delay times and coupling constants for the *t*-butylphenylsilyl and diisopropylsilyl groups. It was not possible to use the INEPT sequence for the diphenylsilyl group because the relaxation time for the phenyl hydrogens is too short. We were aided in the parameter determination by the careful work of Dr. S. Boisvert, who had previously established the optimal parameters for the *t*-butyldimethylsilyl and triisopropylsilyl protecting groups[151].

Using this technique, we were able to get ^{29}Si NMR spectra for the products in which we were most interested, namely the diisopropylsilyl linked nucleotide analogues. In the case of both the dimers and trimers of thymidine and 2'-deoxyadenosine, we saw the expected number of signals, one for the dimers, two for the trimers. These signals (and that for the *t*-butylphenyl derivatives) appear slightly upfield (negative shift) from TMS. The number of signals allowed us to be confident of the number of silyl groups in each molecule, and supported the conclusions drawn from the ^{1}H NMR spectra. Due to the small amount of material available to us, we were not able to obtain acceptable ^{29}Si spectra for the two fully deprotected hexanucleotide analogues.

151. S. Boisvert, Ph. D. Thesis, McGill University, 1987.

Because of the large size of these molecules, the NMR spectra may not be completely acceptable as characterisation. We wished to have yet more evidence of the structures of our products. In spite of repeated attempts, it was not possible to obtain these in crystalline form, in most cases, so that elemental analysis was of dubious value to us. We therefore turned our attention to mass spectrometry. In recent years, fast atom bombardment (FAB) mass spectrometry[152] has been applied to the field of nucleosides and nucleotides as an aid to characterisation and as a possible means of sequencing oligonucleotides. Other forms of mass spectrometry have also been applied to this area[153]. The large size and typically low volatility of nucleotides makes them less than amenable to most of the traditional forms of mass spectral analysis. Some success has been had using the "softer" forms of ionisation, such as chemical ionisation (CI) and FAB. A pair of articles has recently been published describing FAB MS of nucleosides and nucleotides, and comparisons of this technique with electron impact (EI) and CI methods[154]. The FAB technique does not depend upon the volatilisation of the sample. Rather, a beam of fast atoms (in our case, Xe) is directed onto the surface of a liquid in which is dissolved the sample. The solvent (matrix) used here was either diethanolamine or triethanolamine.

In examining our FAB mass spectra, one feature is noticeable. This is that, in the case of the thymidylic acid analogues **96** and **88b**, the molecular ion MH^+ is not seen, but rather one corresponding to MH^+ plus triethanolamine. This is a common characteristic of FAB MS. Solvent adducts are frequently seen, and this may complicate the interpretation of the spectrum. In addition to these adducts, the matrix may itself produce ions corresponding to the molecular ion of the matrix, and increasingly heavy adducts. This may also complicate the spectrum. On the other hand, molecular ions and other large fragments are usually seen, albeit sometimes in the form of adducts. This allows us to get more information than is provided for such molecules by the more common EI and CI routes. The FAB method is also relatively simple to use, and the equipment is adaptable to most common mass spectrometers.

Interestingly, in the case of the 2'-deoxyadenylic acid analogues **104b** and **109** described herein, no solvent adducts to the molecular ion were observed. We have no explanation for this fact. The mechanisms of solvent adduct formation are not clearly understood. In both cases, however, we were able to identify a fragment corresponding to the molecular weight expected for

152. M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Taylor, B. N. Green, *J. Chem. Soc. Chem. Comm.*, 325 (1981).
153. C. J. McNeal, K. K. Ogilvie, N. Y. Theriault, M. J. Nemer, *J. Am. Chem. Soc.*, **104**, 981 (1982).
154. (a) D. L. Slowikowski, K. H. Schram, *Nucleosides & Nucleotides*, **4**, 309 (1985). (b) ----------, ibid., 347 (1985).

the compounds. The reader should note that the mass spectra were obtained in the low resolution mode only.

Attempts to acquire the FAB mass spectra for the fully deprotected hexamers 100 and 114 were unsuccessful. Under the same conditions as used for the fully protected trimers described above, no molecular ion or structurally significant fragments were observed. We also attempted to acquire the mass spectrum of the fully protected oligonucleotide analogue 113. Using triethanolamine, glycerol:pyridine or glycerol:acetic acid as the matrix, no significant ions were seen, except for a small peak at 303 amu (glycerol:acetic acid, glycerol:pyridine), which we assigned to the dimethoxytrityl cation. The absence of any other informative (non-matrix) ions argues neither for nor against the assigned structure. We feel that this particular method is simply not appropriate for use with these larger analogues. Obviously we would have preferred to get mass spectral confirmation of the structural assignment for the hexamers. On the other hand, we feel that the supporting evidence, in particular, the characterisation of the smaller precursors, and the ^{1}H NMR evidence for the fully protected hexamers, is sufficiently strong for us to assign the structure with confidence. The ^{1}H NMR of the fully protected molecules 99 and 113 is particularly useful, showing that both required protecting groups (levulinyl and dimethoxytrityl) are present. The absence of any indication of degradation, the UV and ^{1}H NMR spectra of the final products 114 and 100, the characterisation of the trimers, and the absence of conflicting mass spectral evidence (*i.e.*, evidence of shorter oligomers) all support our conclusions with respect to compounds 100 and 114.

In summary, we feel that the methods used to characterise these molecules were adequate to the task, providing corroborative evidence when compared one with another.

Conclusions

We have described the synthesis and characterisation of a novel class of neutral nucleotide analogues, replacing phosphorus with silicon. Both the stepwise and block condensation methods of synthesis have been used to make the analogues up to six units long. The circular dichroism spectra of the molecules indicate that they are able to assume a fairly rigid, right-handed helix in water solution. This is similar to the helical form assumed by natural nucleotides. The low solubility of these molecules prevented our studying their ability to form duplexes with each other or with natural nucleotides. Degradation studies indicate that base-base interaction occurs in the case of the thymidine series, resulting in a measurable hypochromicity. This is also characteristic of natural nucleotides.

Clearly, we are disappointed that we were unable to carry out hybridisation studies against natural nucleotides. Although our nucleotide analogues are neutral, we felt that it was still

reasonable to expect that they would show appreciable solubility in water. Certainly this has been observed in other cases of neutral nucleotide analogues. Many of the previously synthesised analogues have contained a carbonyl or carbonyl-like oxygen at the internucleotide link. The key to solubilising such molecules may lie in the presence of this oxygen[155]. On the other hand, the ability of our analogues to form helical structures in water solution is encouraging, as it indicates that they are capable of adopting conformations similar to those of their natural counterparts. For further studies to be useful, the solubility problem must be overcome. One method might be to attach solubilising groups to the molecule. Thus a phosphate group might be attached to the 5'- or 3'-terminus of the molecule. Alternatively, one or more phosphate or other groups could be inserted in the body of the molecule. A second method would be to change the nature of the substituents at silicon. We have used alkyl and aryl groups. It may be useful to use some carbonyl-containing group such as a ketone or ester. The former would most likely be preferred as probably being more stable to the deprotection conditions. If such a solubilised silyl nucleotide analog could be synthesised, then hybridisation studies could be undertaken.

If sequences longer than about six units were desired, solid phase synthetic techniques would probably have to be used. This allows one to avoid the tedious and time-consuming separations at every step, which become progressively more difficult as the molecule becomes longer. Coupling yields can be improved by the use of large reagent excesses. This route can provide sufficiently large quantities for hybridisation and optical studies.

In summary, then, we have described a procedure for the synthesis and deprotection of neutral thymidine and 2'-deoxyadenosine nucleotide analogues in which a silicon atom replaces phosphorus in the internucleotide link. A variety of substituents at silicon have been examined, and a range of stability to both acidic and basic conditions noted. The diisopropylsilyl link offers the best combination of reactivity and stability. We have deprotected some of the analogues and showed that the diisopropylsilyl-linked members of the class form stable right-handed helices in water solution, and may exhibit hypochromicity.

155. P. O. P. Ts'o, private communication.

Experimental Section.

Materials and Methods.

Nucleosides were obtained from Boehringer Mannheim Canada (Montreal, Quebec) and Sigma Chemical Co. (St. Louis, Mo.). Silylating agents and dichlorosilane were obtained from Petrarch Systems Inc. (Bertram, Pa.) with the exceptions of bis(trifluoromethanesulphonyl)-diisopropylsilane (Fluka Chemical Corp., Ronkonkoma, N. Y.) and dichlorodiphenylsilane and di-*t*-butyldichlorosilane (Aldrich Chemical Co., Milwaukee, Wi.). These last two were distilled before use. Levulinic acid was obtained from Aldrich and distilled before use. Imidazole (Aldrich) was recrystallised from toluene. Methylene chloride for coupling reactions was distilled from phosphorus pentoxide and stored over activated (450°C) 4A molecular sieves. Dimethylformamide was stirred over calcium hydride and distilled *in vacuo* onto activated 4A molecular sieves. Tetrahydrofuran was predried over these same molecular sieves, before being refluxed with sodium metal and benzophenone until a violet colour persisted. It was freshly distilled from this system just before use. Pyridine was distilled from phthallic anhydride onto activated 4A molecular sieves. Carbon tetrachloride was purified by heating with alcoholic potassium hydroxide, followed by washing with water and distillation from phosphorus pentoxide onto activated 4A molecular sieves. Palladium (II) chloride (Aldrich) was a gift from Dr. A. Shaver. Most other solvents were obtained from Anachemia (Montreal, Quebec) as reagent grade and used as such. Most other reagents were obtained from Aldrich, reagent grade. Deuterated solvents were purchased from Merck, Sharpe, Dohme (Montreal, Quebec) or Aldrich.

Silica gel used for column chromatography was Kieselgel 60 (Merck, Montreal, Quebec), and was used in the ratio of 10-15 g per g of crude material. Preparative silica gel plates were prepared here using Kieselgel 60 GF, 20X20 cm, about 2 mm thick, and used with a maximum loading of 100 mg crude compound plate-1. Bands were scraped off and eluted with ethyl acetate:ethanol, 7:3. Analytical TLC was done on Kieselgel 60 F254 plastic-backed plates, cut to about 8 cm long. Solvent A is diethyl ether:CH_2Cl_2:EtOH, 71:26:3; solvent B is EtOAc:CH_2Cl_2:EtOH, 5:4.5:0.5. All coupling reactions were carried out in Hypovials containing stirring bars, oven-dried (*ca.* 130°C) and cooled in a stream of argon. The vials were sealed with rubber septa. A syringe containing Drierite^{tm} was used to equalise pressure. All reagents in solution were introduced *via* syringe. Such solutions were prepared in sealed, Ar-filled, dry Hypovials. Polypropylene syringes (Aldrich) were used, and vials and syringes were rinsed with 1 or 2 ml of the solvent to complete the transfer. Chloroform:Dry Ice baths were used to attain -

60°C, and acetone or isopropanol:Dry Ice baths for -78°C. ^{1}H and ^{29}Si NMR spectra were obtained using Varian XL-200 and -300 instruments. UV spectra were obtained with Cary 17 and Hewlett-Packard 8451A spectrophotometers. Circular dichroism measurements were made on a JASCO J-500C instrument. Melting points were obtained using a Fisher-Johns apparatus and are uncorrected. FAB mass spectra were run on a Hewlett-Packard 5984A mass spectrometer, using a diethanolamine or triethanolamine matrix at 8 KV and 1 mA (Xe) and are reported as m/z, assignment, relative intensity.

Attempted synthesis of and detritylation of 5'-O-dimethoxytrityl-3'-O-(5'-O-diphenylsilyl-3'-O-monomethoxytritylthymidyl)thymidine 81.

5'-O-dimethoxytritylthymidine **85** (200 mg, .37 mmol, dissolved in DMF, 5 ml) was added dropwise over 10 min to a DMF (5 ml) solution of diphenyldichlorosilane (84 *ul*, .40 mmol) and imidazole (120 mg, 2 mmol). The reaction mixture was stirred at room temperature 10 min, and 3'-O-monomethoxytritylthymidine **80**, R"=MMT (190 mg, .37 mmol) was added, in DMF (5 ml). The reaction was stirred for 1 hour and worked up by pouring into water and extraction with CH_2Cl_2. TLC (CH_2Cl_2:MeOH, 9.5:.5) showed three tritylated materials. Column chromatography yielded the desired **81** (R=DMT, R'=phenyl, R"=MMT) as a soft white foam (64 mg, 29%), m. p. not defined, UV max 266 nm, ^{1}H NMR ($CDCl_3$) 6.35-6.55 (m,2, H1'), 3.72 (m, 9, $-OCH_3$), and the 3',3'-linked dimer **82** (R=DMT, R'=phenyl) (70 mg, 31%, *vide infra*) as the only dimethoxytritylated products (as judged by the colour of the TLC on spraying with 15% aqueous perchloric acid).

Detritylation of **81** was attempted by the use of 3% dichloroacetic acid (DCA) and 3% trichloroacetic acid (TCA) in dichloroethane. A few milligrams of the dimer X were placed in each of two vials. The acid solutions were added (1 ml) to the vials, and the reaction monitored by TLC (CH_2Cl_2:MeOH, 9:1). This showed the production of several materials within 1 minute, in both reactions. One of the products predominated, and comigrated with thymidine on TLC. The use of acetic acid (80% in water) gave the same result within 10 min, while starting material was still present. Detritylation of the 3',3'-linked dimer **82** from the synthesis gave the same result.

Synthesis of 3'-O-levulinylthymidine.

5'-O-dimethoxytritylthymidine **85** (544 mg, 1.0 mmol) was dissolved in anhydrous 1,4-dioxane in a dry 25 ml round-bottom flask fitted with a stirring bar. Dimethylaminopyridine (DMAP, 10 mg, .08 mmol), levulinic acid (.15 ml, 1.5 mmol, 174 mg) and dicyclohexylcarbodiimide

(DCC, .41 g, 2.0 mmol) were added and the reaction mixture allowed to stir at room temperature for 2 h, gradually developing a fine white precipitate. Water was added to destroy excess DCC and the mixture was filtered. The precipitate was washed with CH_2Cl_2 and the organic and aqueous layers were separated. The organic layer was dried with Na_2SO_4 and concentrated. This caused the production of a fine white precipitate, which was removed by filtration. The filtrate was taken up in more CH_2Cl_2, concentrated and filtered again. This step was repeated twice more, at which time the oily product was precipitated from hexanes to give 600 mg (93%) of a white powder, chromatographically pure, m. p. 74-76°C.

The products of several such syntheses were combined for detritylation. Thus 5'-O-dimethoxytrityl-3'-O-levulinylthymidine (4.00 g, 6.2 mmol) was dissolved in acetic acid (80% aqueous, 75 ml) in a 250 ml round-bottom flask. After 1 h at room temperature, *n*-butanol (50 ml) was added to the orange mixture. The solvents were evaporated *in vacuo*, with coevaporation with toluene being used to remove the last traces of acetic acid. The oily residue was purified on a silica gel column ($CHCl_3$:MeOH 1:0 to 9:1 gradient) and recrystallised from toluene to give the desired product **87** as a white powder (1.30 g, 3.8 mmol, 62%), m. p. 113-114°C, UV λmax 268 nm, ^{1}H NMR ($CDCl_3$) 7.49 (s, 1, H6), 6.24 (t, 1, H1'), 5.34 (m, 1, H3'), 4.08 (m, 1, H4'), 3.88 (bs, 2, H5'), 2.76 (t, 2, -$COCH_2CH_2$), 2.56 (t, 2, -$COCH_2CH_2$), 2.20-2.30 (m, 2, H2')2.18 (s, 3, -$COCH_3$), 1.89 (s, 3, CH_3-5), Rf CH_2Cl_2:MeOH, 9:1, .38, solvent A, .20, EtOAc, .19.

Synthesis of 5'-O-dimethoxytrityl-3'-O-(5'-O-diphenylsilyl-3'-O-levulinylthymidyl)thymidine 88b. Preliminary attempts

The following reactions were compared by TLC. The success of the synthesis was judged both by the yield and by the apparent ratio of **88b** to **89b** on TLC. Reactions (a) through (d) were less successful by these standards than reaction (e). These conditions were then used as the standard reaction conditions for further syntheses.

(a) $AgNO_3$ (190 mg, 1.1 mmol) was dissolved in THF (4 ml) and pyridine (.32 ml, 4.0 mmol) in a sealed Hypovial. diphenyldichlorosilane (84 *u*l, .40 mmol) was added. A white precipitate formed. **85** (200 mg, .37 mmol) was dissolved in THF (1.5 ml) and added dropwise over 1 h, with rapid stirring. After the reaction mixture had stirred 1 h at room temperature, **87** (113 mg, .33 mmol) was added, and the mixture was stirred overnight at room temperature. The solids were removed by filtration and the solution concentrated and taken up in CH_2Cl_2, washed twice with water, dried (Na_2SO_4) and the solvent evaporated.

(b) $AgNO_3$ (372 mg, 2.2 mmol) was dissolved in a solution of THF (10 ml) and pyridine (.80 ml, 10 mmol). Diphenyldichlorosilane (232 *u*l, 1.1 mmol) was added, with precipitation. **85**

(544 mg, 1 mmol) was added as a solution in THF (2.0 ml) dropwise over 30 min, at -78°C, with rapid stirring. After 1 h, **87** (308 mg, .9 mmol) was added in THF (2.0 ml) and the reaction mixture left to stir at room temperature overnight. It was then worked up as before. TLC analysis showed that this reaction had the same product distribution as the previous one, so they were combined and purified (silica gel column, CH_2Cl_2:MeOH, 9.5:.5) to yield the desired 3',5'-linked dimer **88b** (417 mg, 31%) and 3',3'-linked dimer **89b** (412 mg, 24%) as the tritylated products.

(c) Diphenyldichlorosilane (84 *ul*, .40 mmol) and imidazole (272 mg, 4.0 mmol) were dissolved in DMF (4 ml). **85** (200 mg, .37 mmol) dissolved in DMF (1.5 ml) was added dropwise over 2 h at room temperature. The reaction mixture was stirred for 1 h, then **87** (113 mg, .33 mmol), dissolved in DMF (1.5 ml) was added, and the mixture stirred at the same temperature overnight. It was worked up by pouring into water and extraction with CH_2Cl_2, then drying and evaporation. TLC showed a poorer product distribution than for the previous two reactions.

(d) The reaction described in the preceding paragraph was repeated, except that the reaction vial was placed in an ice bath until the second nucleoside was added. The same product ratio was observed (TLC).

(e) Dichlorodiphenylsilane (252 *ul*, 1.2 mmol) was dissolved in anhydrous THF (10 ml) in a sealed Hypovial. Anhydrous pyridine (2.4 ml, 30 mmol) was added *via* syringe. The vial was cooled to -78°C in a Dry Ice:acetone bath. 5'-O-dimethoxytritylthymidine **85** (600 mg, 1.1 mmol) was dissolved in anhydrous THF (8 ml) in another vial and added to the silylating mixture *via* syringe dropwise over a period of one hour. The reaction was allowed to stir at -78°C for a further two hours, with the formation of a fine white precipitate. 3'-O-levulinylthymidine (**87**, 340 mg, 1.0 mmol) was added as a solution in anhydrous THF (8 ml). The reaction mixture was then allowed to stir at room temperature overnight. Workup was accomplished by pouring the mixture into water and extracting with CH_2Cl_2. The organic layer was washed once with water, then dried with Na_2SO_4 and evaporated. Coevaporation with toluene removed traces of pyridine, leaving the product mixture as a soft white foam which was dissolved in CH_2Cl_2 and applied to a silica gel column (CH_2Cl_2:solvent A, 1:0 to 0:1 gradient). The desired compound was isolated as a white foam which was precipitated from petroleum ether to yield a white powder (**88b**) (567 mg, 53%). M. p. 113-117°C, UV λmax 265 nm, ^{1}H NMR ($CDCl_3$) 6.25 (m, 1, H1'), 6.48 (m, 1, H1"), 3.77 (s, 6, -O-CH_3), 2.15 (s, 3, -$COCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .45, solvent A, .16, EtOAc, .28.

Deprotection of 88b.

(a) Zinc Bromide Detritylation. **88b** (950 mg, .89 mmol, prepared as described above) was added to a saturated solution of anhydrous $ZnBr_2$ in anhydrous nitromethane (0.1 M, 85 ml,

containing some solid $ZnBr_2$) in a dry 100 ml round-bottom flask with a stirring bar. The solution immediately turned bright orange. After stirring at 0°C for 45 min, the mixture was poured into NH_4OAc(1 M) and extracted with CH_2Cl_2. The organic layer was washed with brine and dried with Na_2SO_4. The solvent was evaporated and the gum coevaporated with toluene to remove nitromethane. Silica gel column chromatography (CH_2Cl_2:MeOH,1:0 to 19:1 gradient) gave the compound **91b**, as a white powder after precipitation from hexanes, in 78% yield (536 mg). M. p 91-93°C, UVλmax 264, ^{1}H NMR 7.45 (m, 10, phenyl) 6.33 (q, 1, H1'), 6.13 (t, 1, H1"), 2.19 (s, 3, $COCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .38, solvent A, .11, EtOAc, .14.

(b) Attempted Catalytic Detritylation. **88b** (300 mg, .28 mmol) was added to a suspension of palladium oxide (30 mg) in cyclohexene (10 ml) and MeOH (20 ml). After refluxing 20 h, TLC (CH_2Cl_2:MeOH, 9:1) showed a very faint trace of **91b**, along with two major products, identical by TLC to thymidine and 3'-levulinylthymidine (**87**). These two products were isolated by chromatography and showed identical ^{1}H NMR characteristics to thymidine and 3'-O-levulinylthymidine. The reaction was repeated using 50 mg **88b** and 5 mg catalyst. After 1 h, TLC showed 3 major products of equal intensity. These corresponded on TLC to the product of zinc bromide detritylation (**91b**, above) and thymidine and 3'-O-levulinylthymidine. This approach was abandoned at this point.

(c) Delevulination. **91b** (536 mg, .70 mmol) was added to a solution of hydrazine hydrate (0.5 M in pyridine:acetic acid, 3:2, 7 ml) in a 50 ml round bottom flask with a stirring bar. After 2.5 min stirring at room temperature, the reaction was quenched by addition of acetyl acetone (21 ml). The reaction was worked up by pouring into water and extraction of the product with CH_2Cl_2. The organic layer was washed with water and dried as above. The solvent was then evaporated and the oily residue coevaporated with toluene to remove traces of pyridine and acetic acid. Column chromatography (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) gave the desired product **92b** (314 mg, 67%) as a white glass. This could not be crystallised. Addition of acetone and evaporation transformed the glass into a foam, which was stirred with ether to give a fine white powder. M. p. 106-109°C, UVλmax 265 nm, ^{1}H NMR (DMSO d-6) 7.50 (bm, 10, phenyl) 6.18-6.26 (m, 2, H1'), Rf CH_2Cl_2:MeOH, 9:1, .25, solvent A, .03, EtOAc, .06.

Catalytic desilylation of 3',5'- and 2',5'-di-O-*t*-butyldimethylsilyluridine 70 and 71.

70 and **71** (100 mg each) were placed in separate flasks. To each was added cyclohexene (7 ml), MeOH (13 ml) and palladium oxide (10 mg). After reflux for 9.5 h, TLC (CH_2Cl_2:MeOH, 9:1) showed complete conversion to the monosilyl derivative. Comparison with standards shows that the products of both reactions move identically on TLC to 2'- and 3'-O-*t*-

butyldimethylsilyluridine (indistinguishable on TLC), and differently from 5'-O-*t*-butyldimethylsilyluridine.

Catalytic detritylation of 3',5'-di-O-dimethoxytritylthymidine.

3',5'-Di-O-dimethoxytritylthymidine (100 mg) was refluxed with MeOH (20 ml), cyclohexene (10 ml) and palladium oxide (13 mg) for 35 min. TLC (CH_2Cl_2:MeOH, 9:1) showed complete conversion to a nontritylated material which migrated with a thymidine standard. Intermediate TLCs run with 3'-O-dimethoxytritylthymidine and 5'-O-dimethoxytritylthymidine standards showed no evidence that one dimethoxytrityl group was removed more rapidly than the other.

Synthesis of 5'-O-dimethoxytrityl-3'-O-(5'-O-diethylsilyl-3'-O-levulinylthymidyl)thymidine 88a.

Diethyldichlorosilane (83 *u*l, .55 mmol) was dissolved in anhydrous THF (25 ml) and anhydrous pyridine (1.2 ml) in a septum-sealed, Ar-filled dry Hypovial fitted with a stirring bar. 5'-O-dimethoxytritylthymidine **85**, dissolved in THF (25 ml) was added dropwise. The coupling reaction was carried out at -78°C, and the addition took 1 hour, as a white precipitate slowly formed. After the reaction was allowed to stir at -78°C for 1.5 h, 3'-O-levulinylthymidine **87** was added in THF solution (4 ml). The reaction was allowed to proceed overnight at room temperature, with stirring. The same workup described above, followed by a silica gel column, (CH_2Cl_2:solvent A, 1:0 to 0:1 gradient) gave the desired 3',5'-linked dimer **88a** in 18% yield (83 mg) along with the 3',3'-linked dimer **89b** in 24% yield (142 mg) as the only tritylated products. M. p. 105-110°C, UVλmax 268 nm, ^{1}H NMR ($CDCl_3$) 6.25-6.35 (bm, 2, H1'), 3.78 (s, 6, -OCH_3), 2.16 (s, 3, -$COCH_3$), .89 (m, 6, -CH_3), .56 (m, 4, -Si-CH_2), Rf CH_2Cl_2:MeOH, 9:1, .45, solvent A, .27, EtOAc, .54.

Synthesis of 5'-O-dimethoxytrityl-3'-O-(5'-O-(*t*-butylmethylsilyl)-3'-O-levulinylthymidyl)thymidine 88d.

Powdered silver nitrate (187 mg, 1.1 mmol) was dissolved in anhydrous THF (5 ml) and pyridine (1.2 ml) in a dry, sealed Hypovial. This required heating with a hair dryer. *t*-Butylmethyldichlorosilane (94 mg, .55 mmol, this reagent is solid at room temperature) was added. A white precipitate immediately formed. 5'-O-dimethoxytritylthymidine **85** (272 mg, .50 mmol) dissolved in THF (4 ml) was added over 30 min, at room temperature. The reaction was allowed to stir at room temperature for 2 h, at which time 3'-O-levulinylthymidine **87** (171 mg, .50 mmol)

dissolved in 2 ml THF, was added, and the reaction left to stir overnight at room temperature. The usual workup and silica gel column (EtOAc) gave the desired product 88d as a pair of diastereoisomers, 135 mg (28% yield) of the faster, and 204 mg (40%) of the slower diastereoisomer as white foams, slightly contaminated (5-10% by TLC) with one another. A small amount of the 3',3'-linked product (89d) was also isolated. Repetition of this synthesis at -78°C gave a yield after purification of 55% desired 88d (both diastereoisomers) and 221 mg (19%) 3',3'-linked dimer 89d. Properties: (1) M. p. 103-108°C, UVλmax 268 nm, ^{1}H NMR ($CDCl_3$) 6.26-6.34 (m, 2, H1'), 3.77 (s, 6, -OCH_3), 2.16 (s, 3, -$COCH_3$), .90 (bs, 9, -CCH_3), .09 (s, 3, -Si-CH_3), Rf CH_2Cl_2:MeOH, 9:1, .31, solvent A, .27, EtOAc, .48, (2) m. p. 106-110°C, UV max 268 nm, ^{1}H NMR ($CDCl_3$) 6.25-6.35 (m, 2, H1'), 3.78 (s, 6, -OCH_3), 2.14 (s, 3, -$COCH_3$), .83 (bs, 9, -CCH_3), .07 (s, 3, -Si-CH_3), Rf CH_2Cl_2:MeOH, 9:1, .45, solvent A, .22, EtOAc, .32.

Synthesis of 5'-O-dimethoxytrityl-3'-O-(5'-O-(phenyl-*t*-butylsilyl)-3'-O-levulinylthymidyl)thymidine 88c.

Powdered silver nitrate (374 mg, 2.2 mmol) was dissolved in anhydrous THF (10 ml) and pyridine (2.4 ml) in a sealed, dry Hypovial. Again, heating was required. *t*-Butylphenyldichlorosilane (232 *u*l, 1.10 mmol) was added, with the formation of a white precipitate. 5'-O-dimethoxytritylthymidine 85 (545 mg, 1.0 mmol) was dissolved in THF (8.0 ml) and added over a period of 30 min at -78°C, with rapid stirring. The cooling bath was removed and the reaction allowed to stir at room temperature for 2 hours. At this point, 3'-O-levulinylthymidine 87 (342 mg, 1.0 mmol), dissolved in THF (4 ml) was added, and the reaction allowed to stir overnight at room temperature. Workup and silica gel column (CH_2Cl_2:solvent A, 1:0 to 0:1 gradient) gave the desired 88c as two diastereoisomers, 147 mg, (14%) of the faster, and 496 mg, (47.5%) of the slower, slightly contaminated with one another. The symmetrically-linked 89c was recovered in 22% yield (276 mg). Properties: (1) m. p. 90-93°C, UV max 266 nm, ^{1}H NMR ($CDCl_3$) 6.23(m, 1, H1'), 6.43 (m, 1, H1"), 3.75 (s, 6, -OCH_3), 2.16 (s, 3, -$COCH_3$), .93 (bs, 9, -CCH_3), ^{29}Si NMR ($CDCl_3$) -19.50, Rf CH_2Cl_2:MeOH, 9:1, .45, solvent A, .31, EtOAc, .45, (2) M. p. 103-110°C, UVλmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.32-6.39 (m, 2, H1'), 3.77 (s, 6, -OCH_3), 2.16 (s, 3, -$COCH_3$), .91 (bs, 9, -CCH_3), ^{29}Si NMR ($CDCl_3$) -20.00, Rf CH_2Cl_2:MeOH, 9:1, .36, solvent A, .16, EtOAc, .32.

Deprotection of 88c (major diastereoisomer).

(a) Detritylation. **88c** (586 mg, .56 mmol) was added to a solution of $ZnBr_2$ (.1 M in nitromethane, 30 ml, with some suspended $ZnBr_2$) at 0°C and the bright orange mixture was stirred 1 h, in the ice bath. TLC (solvent A) after workup showed the reaction to be only about 50% complete. Thus the reaction was repeated on this material. Workup as described (*vide supra*) and silica gel column chromatography (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) gave the desired **91c** as a foam which was precipitated from hexanes (198 mg, 47%). M. p. 106-109°C, UV max 266 nm, ^{1}H NMR ($CDCl_3$) 7.43 (m, 5, phenyl), 6.35 (q, 1, H1'), 6.24 (t, 1, H1''), 2.18 (s, 3, $-COCH_3$), .90 (m, 9, $-CCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .34, solvent A, .11, EtOAc, .15. A further 75 mg (18%) was recovered, slightly contaminated with **88c** (less than 5% by TLC).

(b) Delevulination. **91c**, from the preceding paragraph, (150 mg, .20 mmol) was added to freshly prepared hydrazine solution (*vide supra*) and the reaction stirred at room temperature 5 min. The reaction was quenched by addition of acetyl acetone (7 ml) and worked up as previously described. Precipitation of the crude material from hexane was unsuccessful, as was an attempt to recrystallise from toluene. The desired product **92c** was finally isolated by silica gel column chromatography (CH_2Cl_2:MeOH, 1:0 to 9:1 gradient) and precipitation from hexanes, as a white powder (52 mg, 40%). M. p. 133-140°C, UV λmax 265 nm, ^{1}H NMR (DMSO d-6) 7.45 (m, 5, phenyl), 6.18-6.30 (m, 2, H1'), .92 (s, 9, $-CCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .11, solvent A, .04, EtOAc, .09.

Attempts to synthesise 5'-O-dimethoxytrityl-3'-O(5'-di-*t*-butylsilyl-3'-O-levulinylthymidyl)thymidine 88h.

(a) Di-*t*-butyldichlorosilane (35 *ul*, .15 mmol), imidazole (45 mg. .60 mmol) and 3'-O-levulinylthymidine **87** (50 mg, .15 mmol) were dissolved in DMF (2.0 mmol) in a sealed, dry Hypovial. The solution was stirred at room temperature for 48 h. 5'-O-dimethoxytritylthymidine **85** (81 mg, .15 mmol) was added and the reaction mixture stirred a further 20 h at room temperature. TLC (CH_2Cl_2:MeOH, 9:1) indicated a complex mixture of products. During the usual workup, the appearance of an orange colour suggested detritylation during both the extraction and the attempted purification on two silica gel plates (same solvent). No pure material could be isolated.

(b) $AgNO_3$ (340 mg, 2 mmol) was dissolved with heating in pyridine (2.0 ml) and THF (9.0 ml) in a vial. Di-*t*-butyldichlorosilane (232 *ul*, 1.1 mmol) was added, and a white precipitate slowly formed. **85** (500 mg, .9 mmol) was added as a solution in THF (3.5 ml) and the reaction

stirred at 60°C for 18 h. As TLC showed the starting material **85** consumed, **87** (283 mg, .80 mmol, solution in THF, 3.5 ml) was added and the reaction stirred at room temperature overnight, then at 60°C overnight. TLC indicated that no further reaction had occurred.

(c) **85** (272 mg, .50 mmol) was added to a mixture of $AgNO_3$ (190 mg, 1.1 mmol), pyridine (.40 ml, 5 mmol), di-*t*-butyldichlorosilane (116 *u*l, .50 mmol) and THF (5 ml). A white precipitate formed. The mixture was stirred overnight at 70°C, when TLC (CH_2Cl_2:MeOH, 9:1) showed the starting material **85** had been consumed. 3'-O-dimethoxytritylthymidine **87** (243 mg, .45 mmol) was added, dissolved in THF (1ml), and the reaction stirred at 70°C for 48 h. At this time, none of the second nucleoside **87** had been consumed, and the intermediate noted on TLC was unchanged. This material was not isolated.

Synthesis of diisopropyldichlorosilane.

Magnesium turnings (7.0 g, .29 mol) were placed in a dry 250 ml three-necked flask containing a stirring bar. A few crystals of iodine and anhydrous diethyl ether (15 ml) were added. The flask was fitted with a drying tube and a dropping funnel. 2-Bromopropane (24 ml, 31 g, 25 mol) dissolved in anhydrous diethyl ether (110 ml) was added dropwise *via* the dropping funnel, with rapid stirring, at a rate which was just rapid enough to allow the solvent to reflux gently. The mixture turned dark and cloudy. After the addition was complete, the dropping funnel was replaced with a condenser and the mixture refluxed one hour, then filtered through glass wool into a 500 ml three-necked flask. Meanwhile, dichlorosilane (12 ml, 14.6 g, .15 mol) was condensed into a graduated cylinder. This cylinder was sealed with a septum pierced by a Pasteur pipette which was connected by Tygon tubing to the dichlorosilane cylinder. The graduated cylinder was fitted with a drying syringe and placed in a Dry Ice:acetone bath. The dichlorosilane was allowed to flow through the tube and into the graduated cylinder, where it condensed. The Tygon tubing was then disconnected from the tube and connected to a gas inlet tube fitted to the 500 ml flask containing the Grignard reagent. This flask was also fitted with a stirring bar and a Dry Ice condenser. The cooling bath around the graduated cylinder was removed and the dichlorosilane allowed to distill gently into the rapidly stirred Grignard solution. The addition took about 3.5 h, during which time the addition had to be stopped briefly several times to clear a thick precipitate from the gas inlet tube. During these interruptions, the cooling bath around the graduated cylinder was replaced to avoid loss of the reagent. The mixture was refluxed for 1 h, then poured slowly into cold 10% aqueous acetic acid. The organic layer was washed 5 times with water, at which time the washings were neutral to litmus paper. The solvent volume was reduced by

evaporation in a stream of Ar, then the product was distilled at atmospheric pressure. The desired product, diisopropylsilane, was isolated as a clear colourless liquid, in 46% yield (6.76 g, b. p. 98-100°C, lit. b. p. 98.5°C[156]).

This product was placed in a steel bomb with carbon tetrachloride (23 ml; .24 mol). Palladium (II) chloride (106 mg, 0.6 mmol) was added. This addition was accompanied by very rapid heating and bubbling of the reaction mixture. The bomb was sealed and heated at 140°C for 8 h, then allowed to cool overnight. The reaction mixture was removed by Pasteur pipette as a cloudy brown mixture. The bomb was rinsed with anhydrous ether, and the mixture and washings combined. Solids were removed by centrifugation, and the solvent evaporated in a stream of Ar The oily residue was distilled *in vacuo*, to yield the desired product diisopropyldichlorosilane (4.45 g, 41%, b. p 50-55°C at 5 mm Hg, lit. b. p. 67-69°C at 11 mm Hg[157]) as a clear colourless liquid. ^{29}Si NMR ($CDCl_3$) 38.15 (no lit value available, corresponding value for di-*t*-butyldichlorosilane 39.10[158]).

Synthesis and deprotection of 5'-O-dimethoxytrityl-3'-O-(5'-diisopropylsilyl-3'-O-levulinylthymidyl)thymidine 88e.

(a) 5'-O-dimethoxytritylthymidine **85** (816 mg, 1.50 mmol) was dissolved in CH_2Cl_2 (24 ml), and added dropwise *via* syringe to a solution of the silylating agent bis(trifluoromethanesulphonyl)diisopropylsilane **95** (486 µl, 1.65 mmol) and pyridine (3.6 ml) in CH_2Cl_2 (5 ml). The addition took place over 30 min at -78°C with rapid stirring The reaction mixture was allowed to stir at -78°C for 30 min, then at room temperature for an additional 30 min. 3'-O-levulinylthymidine **87** (513 mg, 1.50 mmol) was added as a solid, and the reaction allowed to stir 1 h at room temperature. The usual workup and silica gel column (CH_2Cl_2 solvent A, 1:0 to 0:1 gradient) yielded the desired product **88e** as a soft white foam (679 mg, 47%) M p not defined, UV λmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.24-6.40 (m, 2, H1'), 3.77 (s, 6, -OCH_3), 2.14 (s, 3, -$COCH_3$), .99 (m, 14, -Si-$CH(CH_3)_2$), ^{29}Si NMR (CDCl3) -7.52, Rf CH_2Cl_2 MeOH, 9:1, .48, solvent A, 27, EtOAc, 47 Mass spectral data (triethanolamine matrix) m/z 1146.5, MH^+ + triethanolamine, rel. int. 100 The symmetrically linked **89e** was also isolated (202 mg, 11%).

(b) **85** (2.31 g, 4.23 mmol) was dissolved in CH_2Cl_2 (50 ml) and added dropwise over 30 min at -78°C to a CH_2Cl_2 (10 ml) solution of bis(trifluoromethanesulphonyl)diisopropylsilane **95** (1.00 ml, 3.39 mmol) and pyridine (8.0 ml) The reaction mixture was stirred at -78°C 30 min and

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157. C.D. Hurd, W. A. Yarnall, *J. Am Chem. Soc*, 71, 755 (1949).
158. T. A. Blinka, B. J. Helmer, R. West, *Adv Organomet Chem.*, 23 (1984)

room temperature 30 min. **87** (724 mg, 2.12 mmol) was added in CH_2Cl_2 (10 ml) and the reaction mixture left to stir overnight at room temperature. Workup and chromatography gave the desired dimer **88e** in 74% yield.

Detritylation was accomplished by adding the product **88e** (679 mg, .70 mmol) to trichloroacetic acid (3% w/v in CH_2Cl_2, 20 ml) and allowing the bright orange solution to stir at room temperature for 10 min. The reaction mixture was then poured into 5% aqueous sodium bicarbonate, the organic layer dried with Na_2SO_4 and concentrated *in vacuo*. The desired product **91e** was purified on a silica gel column (CH_2Cl_2:MeOH 1:0 to 19:1 gradient) and isolated in 85% yield (400 mg), again as a white foam. A trace (*ca.* 20 mg) unreacted **88e** was also recovered. M p. undefined, UVλmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.30 (q, 1, H1'), 6.19 (t, 1, H1''), 2.19 (s, 3, -$COCH_3$), .90 (m, 14, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .20, solvent A, .11, EtOAc, .19.

Deleveulination was accomplished by addition of the detritylated dimer **91e** (170 mg, .24 mmol) to hydrazine hydrate (.5 M in pyridine:acetic acid, 3.2:2.5 ml). The reaction mixture was stirred at room temperature for 6 min, then acetyl acetone (8 ml) was added to consume unreacted hydrazine. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and dried (Na_2SO_4). The solvent was evaporated and the residue coevaporated with toluene. The gummy residue was dissolved in CH_2Cl_2 and applied to two preparative silica gel plates, which were developed in CH_2Cl_2.MeOH, 9:1. The product was removed from the plates, dissolved in CH_2Cl_2:MeOH, 9 1 and precipitated from hexanes, to yield the fully deprotected dimer **92e** as an off-white powder in 64% yield (93 mg). M. p. 104-108°C, UVλmax 266 nm, ^{1}H NMR (DMSO-d_6) 6.19 (m, 2, H1'), 1.01 (bs, 14, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9.1, .07, solvent A, .03, EtOAc, .08.

Synthesis of 5'-O-dimethoxytrityl-3'-O-((5'-O-diisopropylsilylthymidyl-3'-O-(5'-O-diisopropylsilyl-3'-O-levulinylthymidyl))thymidine 96.

(a) 5'-O-dimethoxytritylthymidine **85** (188 mg, .345 mmol) was dissolved in CH_2Cl_2 (3 ml) and added dropwise *via* syringe to a solution of bis(trifluoromethanesulfonyl)diisopropylsilane **95** (102 *u*l, .35 mmol) and pyridine (1.0 ml) in CH_2Cl_2 (3 ml). The addition took place over 30 min at -78°C, with rapid stirring. After 30 min at this temperature and 1 h at room temperature, **91e** (240 mg, .345 mmol) was added as a solution in CH_2Cl_2 (5 ml). The reaction was allowed to stir at room temperature overnight, turning slightly orange. The usual workup and silica gel column (0 to 5% MeOH in CH_2Cl_2) gave the desired trimer **96** in 55% yield (235 mg) as a foam. **89b** (151 mg, 36%) was also isolated. **96** m. p. 103-106°C, UV λmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.19-6.39 (bm, 3, H1'), 3.76 (s, 6, -OCH_3), 2.19 (s, 3, -$COCH_3$), 1.00 (m, 28, -Si-$CH(CH_3)_2$), ^{29}Si NMR

($CDCl_3$) -7.64, -7.81, Rf CH_2Cl_2:MeOH, 9:1, .38, solvent A, .07, EtOAc, .18. Mass spectral data (triethanolamine matrix) 1500.5, MH^+ + triethanolamine, rel. int. 58.04.

(b) **85** (1.09 g, 2.0 mmol), dissolved in CH_2Cl_2 (25 ml) was added dropwise to a solution of bis(trifluoromethanesulphonyl)diisopropylsilane (470 *u*l, 1.6 mmol) and pyridine (.5 ml) in CH_2Cl_2 (5 ml). The addition took place over 30 min at -78°C. The reaction mixture was allowed to stir at this temperature for 30 min, then at room temperature for 30 min. **91e** (900 mg, 1.30 mmol) was then added as a solid, and the reaction mixture stirred 1 h at room temperature. Workup and chromatography as outlined above gave the desired trimer **96** in 68% yield.

Partial deprotection of 96. Preparation of partially protected trimer blocks.

(a) Detritylation. **96** (284 mg, .23 mmol) was treated with trichloroacetic acid (3% CH_2Cl_2 solution, 6.2 ml) for 5 min at room temperature. Neutralisation with 5% aqueous sodium bicarbonate and drying of the organic layer with Na_2SO_4 was followed by silica gel column purification (:CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) which yielded the partially protected trimer **97** as a foam (157 mg, 73%). M. p. 96-100°C, UVλ max 270 nm, ^{1}H NMR ($CDCl_3$) 6.24 (m, 2, H1'), 6.13 (t, 1, H1"), 2.17 (s, 3, -$COCH_3$), 1.88 (bs, 9, -CH_3), 1.03 (m, 28, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .34, solvent A, .04, EtOAc, .11.

(b) Delevulination. **96** (284 mg, .23 mmol) was treated with hydrazine solution (.5 M in pyridine:acetic acid, 3.2, 2.3 ml) at room temperature for 10 min. Acetyl acetone (9 ml) was added and the reaction worked up by pouring into water and extraction with CH_2Cl_2. The organic layer was dried, concentrated and coevaporated with toluene before a silica gel column (same solvent as above) and precipitation from hexanes gave the desired **98** in 88% yield (231 mg). M. p. 110-113°C, UVλmax 268 nm, ^{1}H NMR ($CDCl_3$) 6.22-6.36 (bm, 3, H1'), 3.77 (s, 6, -OCH_3), 1.87, 1.84, 1.51 (s, 3, -CH_3), .98 (bm, 28, -Si-$CH(CH_3)_2$); Rf CH_2Cl_2:MeOH, 9:1, .33, solvent A, .06, EtOAc, .18.

Synthesis and deprotection of the thymidine hexanucleotide analog 99.

Bis(trifluoromethanesulfonyl)diisopropylsilane (55 *u*l, 19 mmol) was dissolved in CH_2Cl_2 (1.5 ml) and pyridine (.5 ml) in a dry, sealed Hypovial. 5'-O-dimethoxytrityl-3'-O-(5'-O-diisopropylsilylthymidyl-3'-O-(5'-O-diisopropylsilylthymidyl))thymidine **98** (194 mg, .21 mmol), dissolved in CH_2Cl_2 (3 ml) was added dropwise over 30 min, at -78°C. The reaction mixture was then allowed to stir at -78°C for 75 min, then at room temperature for a further 75 min. At this point, 3'-O-((5'-O-diisopropylsilylthymidyl-3'-O-(5'-O-diisopropylsilyl-3'-O-

levulinylthymidyl))thymidine **97** (157 mg, .17 mmol), dissolved in CH_2Cl_2 (5 ml) was added and the reaction mixture left to stir overnight (16 h). The solution turned slightly orange during this time. The usual workup was followed by purification on a silica gel column (CH_2Cl_2:solvent B 1.0 to 0:1 gradient) and careful repurification on two silica gel plates (solvent B, 2 developments) to yield after elution, 50 mg compound **99** as a gum. This corresponds to 12% yield. TLC of the reaction mixture indicated that the desired product comprised at least 50% of the tritylated products of the reaction. M.p. not defined, UV max 268 nm, ^1H NMR ($CDCl_3$) 6.4 (bm, 6, H1'), 3.78 (s, 6, -OCH_3), 2.18 (s, *ca.* 3, -$COCH_3$), 1.00 (bs, *ca.* 70, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .42, solvent A, .03, EtOAc, .12.

Deprotection was carried out immediately. Detritylation was accomplished by treating compound **99** (50 mg, .025 mmol) with trichloroacetic acid (1 ml) for 10 min, followed by the usual workup. The crude material was delevulinated with NH_4OH:MeOH, 4:1 (5 ml, mixed with 3 ml dioxane for solubility) for 16 h at room temperature. The mixture of solvents was evaporated, and the crude compound was purified on a silica gel column (CH_2Cl_2:MeOH, 9:1) to yield the desired fully deprotected product **100** as the only nucleosidic product (14 mg, 35%) in the form of a slightly yellow oil UV λmax, 268 nm, ^1H NMR ($CDCl_3$) 6.10-6.31 (bm, 6, H1'), 1.06 (m, *ca.* 70, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .07, solvent A, .03, EtOAc, .07.

Reaction of N,N-dimethylaminodiphenylsilyl chloride with 85.

N,N-dimethylaminodiphenylsilyl chloride (78 *ul*, .40 mmol) was dissolved in anhydrous THF (5 ml) and pyridine (0.4 ml) in a sealed, dry Ar-filled Hypovial. **85** (200 mg, .37 mmol) was added in one portion, and the slightly cloudy reaction mixture allowed to stir at room temperature for 2.5 h. It was then poured into saturated aqueous sodium bicarbonate solution and extracted with CH_2Cl_2. The organic layer was washed once with water, evaporated and coevaporated with toluene. The resulting white foam, nearly pure by TLC, comigrated with symmetrical dimer **89b**, and was identical to **89b** in its ^1H NMR. The reaction was repeated using N,N-diisopropylethylamine as base (.65 ml, 3.7 mmol), with the same result. The reaction was again repeated, this time with the addition of silver nitrate (56 mg, .40 mmol, white precipitate forms on addition of the silylating agent), again using diisopropylethylamine as base. This time the nucleoside **85** was dissolved in THF (1.5 ml) and added to the silylating mixture dropwise *via* syringe over 5 min. The reaction was allowed to stir at room temperature 1 h, before being filtered and worked up as before. The same product was obtained.

Synthesis of 3',3'-linked symmetrical thymidine dimers 89.

(a) R=ethyl. 85 (545 mg, 1.0 mmol) was dissolved in anhydrous THF (10 ml) and pyridine (1.2 ml). Diethyldichlorosilane (83 *u*l, .55 mmol) was added and the reaction stirred at room temperature 1.5 h, as a white precipitate formed. The reaction mixture was poured into brine and extracted with CH_2Cl_2. The organic layer was dried with Na_2SO_4, reduced in volume and coevaporated with toluene. Silica gel column purification (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) gave **89a** in 70% yield (409 mg) as a white powder after precipitation from petroleum ether. M p 115-118°C, UVλmax 268 nm, ^{1}H NMR ($CDCl_3$) 6.37 (t, 2, H1'), 3.75 (s, 12, -OCH_3), .83 (m, 6, -CH_3), .47 (m, 4, -Si-CH_2-), Rf CH_2Cl_2 MeOH, 9:1, 60, solvent A, .54, EtOAc, .76.

(b) R=cyclotetramethylene. The reaction above was repeated with cyclotetramethylenedichlorosilane (72 *u*l, .55 mmol) as the silylating agent. The reaction was identical in all other respects. The chromatography solvent was CH_2Cl_2 EtOAc, 1:0 to 1:1 gradient. The product **89g** was isolated as a soft white foam (355 mg, 60 %) M p not defined, UVλmax 270 nm, ^{1}H ($CDCl_3$) 6.36 (t, 2, H1'), 3.75 (s, 12, -O-CH_3), 1.50 (bs, 4, -CH_2-), .45 (bs, 4, -Si-CH_2-), Rf CH_2Cl_2:MeOH, 9:1, 46, solvent A, .48, EtOAc, .36.

(c) R=*t*-butyl, phenyl. Silver nitrate (187 mg, 1.1 mmol) was dissolved in anhydrous THF (10 ml) and pyridine (1.2 ml) 85 (545 mg, 1 mmol) was added, followed by *t*-butylphenyldichlorosilane (116 *u*l, .55 mmol) A white precipitate formed over a few minutes, and the sealed reaction vial was placed in a 50°C bath for 24 h. Filtration, followed by the usual workup and silica gel column (as in (b)) gave **89c** (328 mg, 52%) as a white foam. M p 130-135°C, UVλ268 nm, ^{1}H NMR ($CDCl_3$) 6.40 (m, 2, H1'), 3.75 (s, 12, -OCH_3), .88 (bs, 9, -$C(CH_3)_3$), ^{29}Si NMR ($CDCl_3$) -22.53, Rf CH_2Cl_2:MeOH, 9:1, 49, solvent A, 57, EtOAc, 77.

(d) R=methyl The synthesis of paragraph (a) was repeated with dimethyldichlorosilane (67 *u*l, .55 mmol) as silylating agent. Workup and column purification gave the product **89f** in 22 % yield as a foam. M. p. 115-122°C, UV max 268 nm, ^{1}H NMR ($CDCl_3$) 6.40 (m, 2, H1'), 3.75 (s, 12, -OCH_3), 0.00 (s, 6, -Si-CH_3), Rf CH_2Cl_2:MeOH, 9:1, .38, solvent A, .46, EtOAc, 72.

(e) R=phenyl. As for paragraph (a), using diphenyldichlorosilane (115 *u*l, .55 mmol). The product **89b** was isolated in 47% yield (300 mg, white foam). M. p. 135-140°C, UVλ max 270 nm, ^{1}H NMR ($CDCl_3$) 6.50 (m, 2, H1'), 3.75 (s, 12, -OCH_3), and large multiplet *ca.* 7.4 ppm unresolved from trityl and base protons, Rf CH_2Cl_2:MeOH, 9:1, .39, solvent A, .46, EtOAc, .79.

(f) R=*t*-butyl, methyl. As for paragraph (c), with *t*-butylmethyldichlorosilane (94 mg, .55 mmol).The product **89d** was isolated a white foam (365 mg, 62 %). M. p. 121-126°C, UVλmax

270, ^{1}H NMR ($CDCl_3$) 6.36 (m, 2, H1'), 3.75 (s, 12, -OCH_3), .80 (s, 9, -$C(CH_3)_3$), 0.0 (s, 3, -Si-CH_3), Rf CH_2Cl_2:MeOH, 9:1, .63, solvent A, .53, EtOAc, .74.

(g) R=isopropyl. As for (a) with bis(trifluoromethanesulphonyl)diisopropylsilane (163 *u*l, .55 mmol). The product **89e** was isolated in 36% yield (217 mg) after chromatography and precipitation from hexanes. M. p. 112-115^{o}C, UVλmax 268, ^{1}H NMR ($CDCl_3$) 6.37 (m, 2, H1'), 3.75 (s, 12, -OCH_3), .90 (m, 14, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .45, solvent A, .51, EtOAc, .57.

Synthesis of 5',5'-linked symmetrically linked thymidine dimers 90.

(a) R=ethyl. The reaction of paragraph (a) above was repeated using 3'-O-levulinylthymidine **87** (342 mg, 1.0 mmol) as the nucleoside. The same workup and column purification yielded **90a** as a white gummy material (267 mg, 75%). M. p. not defined, UVλ max 264 nm, ^{1}H NMR ($CDCl_3$) 6.30 (m, 2, H1'), 2.17 (s, 6, -$COCH_3$), 1.00 (t, 6, -CH_3), .72 (m, 4, -Si-CH_2-).

(b) R=cyclotetramethylene. The reaction of paragraph (a) was repeated with cyclotetramethylenedichlorosilane (72 *u*l, .55 mmol) as the silylating agent. The product **90g** was isolated in 26% yield as a gum. M. p. not defined, UVλmax 265, ^{1}H NMR ($CDCl_3$) 6.33 (q, 2, H1'), 2.17 (s, 6, -$COCH_3$), 1.58 (bs, 4, -CH_2-CH_2-), .60 (bs, 4, -Si-CH_2-).

(c) R=*t*-butyl, phenyl. The coupling described above was carried out with *t*-butylphenyldichlorosilane (116 *u*l, .55 mmol) and silver nitrate (187 mg, 1.1 mmol). A white precipitate formed. The reaction time was 21 h, at a temperature of 50^{o}C. The product **90c** was recovered as a white powder (310 mg, 73%) after chromatography and precipitation from hexanes M. p. less than 80^{o}C, UVλmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.35 (m, 2, H1'), 2.15 (s, 6, -$COCH_3$), .90 (bs, 9, -$C(CH_3)_3$), ^{29}Si NMR ($CDCl_3$) -17.99.

(d) R=methyl. The reaction described in (a) was repeated using dimethyldichlorosilane (67 *u*l, .55 mmol) as the silylating agent. The same workup and purification gave the desired compound **90f** as a soft white foam in 30% yield (108 mg). M. p. not defined, UVλmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.35 (m, 2, H1'), 2.19 (s, 6, -$COCH_3$), .22 (2, 6, -Si-CH_3).

(e) R=phenyl. As for (a), using diphenyldichlorosilane (115 *u*l, .55 mmol). The yield was only 15% (64 mg) of a white gummy material (**90b**). M. p. not defined, UVλmax 270 nm, ^{1}H NMR ($CDCl_3$) 7.4 (bm, 10, phenyl) 6.34 (q, 2, H1'), 2.18 (s, 6, -$COCH_3$).

(f) R=*t*-butyl, methyl. The reaction as described in (c) using *t*-butylmethyldichlorosilane (94 mg, .55 mmol) gave the product **90d** in 62% yield (243 mg) as a white foam. M. p. not defined, UVλmax 266 nm, ^{1}H NMR ($CDCl_3$) 6.32 (m, 2, H1'), 2.18 (s, 6, -$COCH_3$), .97 (bs, 9, -$C(CH_3)_3$).

(g) R=isopropyl. As for (a) with bis(trifluoromethanesulphonyl)diisopropylsilane (163 *ul*, .55 mmol), with the product **90f** isolated in 35% yield (140 mg) of a white powder after precipitation from hexanes. This powder quickly softened to a gummy consistency. M. p. not defined, UVλ max 264 nm, ^{1}H NMR ($CDCl_3$) 6.35 (m, 2, H1'), 2.20 (s, 6, -$COCH_3$), 1.10 (bs, 14, -Si-$CH(CH_3)_2$).

Attempts to synthesise 5'-O-dimethoxytrityl-3'-O-(5'-diisopropylsilyl-3'-O-levulinylthymidyl)thymidine 88e using diisopropyldichlorosilane.

Diisopropyldichlorosilane (98 *ul*, .55 mmol, 104 mg, prepared as described above) was dissolved in anhydrous THF (5 ml) and pyridine (1.2 ml) in a sealed Hypovial containing a stirring bar. **85** (300 mg, 55 mmol) dissolved in THF (4 ml) was added dropwise *via* syringe over a period of 10 min at 0°C, with rapid stirring. After 15 min, the cooling bath was removed and the reaction mixture was left at room temperature for 1.5 h. TLC (CH_2Cl_2:MeOH, 9.1) indicated that no reaction had occurred. The vial was placed in a 50°C bath for 24 h. TLC in the same solvent system showed only about 50% of the starting material had been consumed.

The above experiment was repeated using **87** as the nucleoside. TLC indicated the same result, with only about 50% reaction after 24 h at elevated temperature.

85 (81 mg, .15 mmol) was dissolved in DMF (2 ml) and added dropwise over 30 min to a solution of diisopropyldichlorosilane (26 *ul*, .15 mmol) and imidazole (45 mg, .60 mmol) in anhydrous DMF (1 ml) at -78°C. The reaction mixture was stirred at this temperature for 30 min, then the bath was removed and stirring continued overnight. **87** (50 mg, .15 mmol) was added and the reaction left at room temperature a further 24 h. The reaction was worked up as usual The TLC showed mainly the symmetrical dimers **89e** and **90e**, with very little of the desired 3',5'-linked **88e**.

Silver nitrate (25 mg, 15 mmol) was dissolved in THF (2.0 ml) and pyridine (5 ml) This was added dropwise over a few minutes to a vial containing diisopropyldichlorosilane (26 *ul*, 15 mmol) at -78°C, with rapid stirring. **85** (81 mg, .15 mmol) in THF (2 ml) was added dropwise over 15 min at the same temperature. By this time, a white precipitate had formed. The reaction mixture was stirred 15 min, then the bath was removed and the stirring continued 14 h at room temperature. **87** (50 mg, .15 mmol) was added as a solid, along with silver nitrate (25 mg, .15 mmol). After 2 h a further 25 mg silver nitrate was added The reaction was filtered and worked up as usual 2 h later. TLC (solvent A) showed mainly **89e** and **90e**, as above.

This experiment was repeated, this time dissolving the silver nitrate with the first nucleoside **85**, and adding dropwise to the solution of silylating agent and pyridine in THF (.5 ml)

The reaction was worked up 24 h after the second nucleoside **87** was added. No improvement (TLC) was noted.

Synthesis of 2'-deoxy-3'-O-levulinyladenosine 102.

5'-O-dimethoxytrityl-2'-deoxyadenosine **101**[159] (1.95 g, 3.5 mmol) was dissolved in anhydrous 1,4-dioxane (20 ml) in a dry round bottom flask containing a stirring bar. Levulinic acid (4.0 ml, .45 g, 3.9 mmol) and DMAP (47 mg, .35 mmol) were added, followed by DCC (2.2 g, 10.5 mmol). The reaction was stirred for 2 h, with formation of a white precipitate, when TLC (CH_2Cl_2:MeOH, 9:1) showed all starting material had been consumed. Water was added, and the reaction mixture stirred a few minutes before filtration and worked up as usual. DCU was removed by chromatography on a short silica gel column (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient). The gummy yellow product was treated with acetic acid (80%) for 1 h, at room temperature, with stirring. *n*-Butanol was added to the bright orange solution until the color had faded, then solvents were evaporated at reduced pressure. The residue was coevaporated with toluene to remove acetic acid, and crystallised from toluene to yield **102** (852 mg, 70% overall yield) as a white powder, m. p. 147-150°C, UVλmax 260 nm, ^{1}H NMR ($CDCl_3$) 8.32, 8.16 (s, 2, H2, H8), 6.46 (t, 1, H1'), 5.49 (m, 1, H3'), 4.20 (bs, 1, H4'), 4.03 (bd, 2, H5', H5''), 2.56-2.80 (m, 6, H2', H2'', -CH_2-CH_2), 2.18 (s, 3, -$COCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .35, solvent A, .00, EtOAc, .04.

Attempted synthesis of 5'-O-dimethoxytrityl-2'-deoxy-3'-O-(5'-O-diisopropylsilyl-2'-deoxy-3'-O-levulinyladenylyl)adenosine 104c using bis(trifluoromethanesulphonyl)diisopropylsilane.

5'-O-dimethoxytrityl-2'-deoxyadenosine **101** (227 mg, .50 mmol) was dissolved in anhydrous CH_2Cl_2 (8 ml) and added dropwise, over 1 h, to a solution of the silylating agent (95, 162 *u*l, .55 mmol) in CH_2Cl_2 (5 ml) and pyridine (1.2 ml), at -78°C. The reaction mixture was allowed to stir at room temperature 2 h. 3'-O-levulinyl-2'-deoxyadenosine **102** (154 mg, .50 mmol) was added as a solid and the reaction left to stir overnight at room temperature. The usual workup was carried out. TLC (CH_2Cl_2:MeOH, 9:1) showed a complex mixture of products, none of which predominated. Attempts to purify this mixture on a silica gel column (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) were unsuccessful, resulting in mixtures of products.

159. G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.*, **104**, 1316 (1982).

Synthesis of N^6-benzoyl-2'-deoxy-3'-O-levulinyladenosine 108.

5'-O-dimethoxytrityl-N^6-benzoyl-2'-deoxyadenosine **107** (2.0 g, 3.04 mmol) was dissolved in anhydrous 1,4-dioxane (30 ml) in a dry round-bottom flask fitted with a stirring bar. Levulinic acid (.40 ml, 45 mg, 3.9 mmol) and a few milligrams of DMAP were added, followed by DCC (1.40 g, 6.7 mmol). A white precipitate formed as the reaction mixture stirred at room temperature for 1 h. Water was added and the reaction mixture filtered and worked up as usual. The crude product was treated with trichloroacetic acid (3% w/v in CH_2Cl_2, 100 ml) for 5 min. The bright orange solution was poured into 5% aqueous sodium bicarbonate and extracted with CH_2Cl_2. After drying (Na_2SO_4) and evaporation of the solvent, the desired product **108** was crystallised from toluene as a white powder, pure by TLC, in 69% yield (946 mg). M. p. softens 105-110°C, melts 145-147°C, UV 284 nm, ^{1}H NMR ($CDCl_3$) 8.79, 8.09 (s, 1, H2, H8) 6.37 (q, 1, H1'), 5.56 (dd, 1, h3'), 4.30 (bs, 1, H4'), 3.95 (bs, 2, H5'), 3.19 (m, 1, H2'), 2.82 (t, 2, -CH_2-), 2.47-2.64 (m, 4, H2", -CH_2-), 2.22 (s, 3, -$COCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .47, solvent A, .07, EtOAc, .09.

Synthesis of bis(5'-O-dimethoxytrityl-2'-deoxyadenosine)diphenylsilane.

101 (200 mg, .36 mmol) was dissolved in THF (5.0 ml) and pyridine (0.9 ml). Diphenyldichlorosilane (42 *ul*, .20 mmol) was added and the reaction stirred at room temperature 3 h. The usual workup and silica gel column (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) gave the desired product as a white foam (105 mg, 46 % yield). M. p. 105-108°C, UV 262 nm, ^{1}H NMR ($CDCl_3$) 6.44 (t, 2, H1'), 3.74 (s, 12, -OCH_3), Rf CH_2Cl_2:MeOH, 9:1, .14, solvent A, .00, EtOAc, .03.

Synthesis of 2'-deoxy-5'-O-dimethoxytrityl-3'-O-(5'-diphenylsilyl-2'-deoxy-3'-O-levulinyladenylyl)adenosine 104a.

Diphenyldichlorosilane (252 *ul*, 1.2 mmol) was dissolved in THF (10 ml) and pyridine (2.4 ml). **101** (609 mg, 1.1 mmol) was dissolved in THF (8 ml) and added to the silylating mixture dropwise *via* syringe over 30 min at -78°C, with rapid stirring. After the reaction had been allowed to run 2 h, **102** (350 mg, 1.0 mmol) was added as a solution in THF (8 ml). The cooling bath was removed and the reaction allowed to stir at room temperature overnight. The usual workup and column (CH_2Cl_2:MeOH, 1:0 to 9:1 gradient) gave the desired product **104a** in 45 % yield (490 mg) as a foam. M. p. 82-85°C, UV λmax 262 nm, ^{1}H NMR ($CDCl_3$) 7.4 (m, unresolved from trityl and base resonances), 6.55 (t, 1, H1'), 6.41 (q, 1, H1"), 3.75 (s, 6, -OCH_3), 2.16 (s, 3, -

$COCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .09, solvent A, .00, EtOAc, .00. The expected symmetrically linked dimers were also isolated.

Deprotection of 5'-O-dimethoxytrityl-3'-O-(5'-diphenylsilyl-3'-O-levulinyladenylyl)adenosine 104a.

(a) Detritylation. **104a** (420 mg, 39 mmol) was added to a solution of zinc bromide (saturated, in nitromethane, 25 ml) at 0°C and allowed to react for 75 min. At the end of this period, the bright orange reaction mixture was poured into 1M ammonium acetate and worked up as usual. The desired product 105a was isolated by precipitation from hexanes as a white powder (260 mg, 86%). ^{1}H NMR ($CDCl_3$) 8.28, 8.26, 8.01, 7.90 (s, 4, H2, H8), 7.4-7.6 (bm, 10 phenyl), 6.40 (m, 2, H1'), 2.20 (s, 3, -$COCH_3$), Rf CH_2Cl_2:MeOH, 9:1, .07, solvent A, 00, EtOAc, .00.

(b) Delevulination. The product (105a) of the previous reaction was added to hydrazine solution (3.5 ml) and allowed to react at room temperature for 3 min. Acetyl acetone (2.5 ml) was added, and the reaction worked up as usual. Silica gel column chromatography (CH_2Cl_2:MeOH, 19:1 to 9:1 gradient) gave the desired product **106a** as a chromatographically pure white foam (141 mg, 62%). M. p. 96-99°C, UV λmax 262 nm, ^{1}H NMR (DMSO-d_6) 8.29, 8.21, 8.09, 8.05 (s, 4, H2, H8), 7.3-7.6 (bm, 10, phenyl), 6.45 (m, 1, H1'), 6.34 (H1''), Rf CH_2Cl_2:MeOH, 9:1, .16, solvent A, .00, EtOAc, .00.

Synthesis of N^6-benzoyl-2'-deoxy-5'-O-dimethoxytrityl-3'-O-(5'-O-diisopropylsilyl-N^6-benzoyl-2'-deoxy-3'-O-levulinyladenylyl)adenosine 104b.

(a) N^6-benzoyl-5'-O-dimethoxytrityl-2'-deoxyadenosine (107, 737 mg, 1.12 mmol) was dissolved in anhydrous DMF (8.5 ml) and added dropwise over 30 min to a solution of diisopropyldichlorosilane (216 ul, 1.24 mmol) and imidazole (763 mg, 11.2 mmol) in DMF (4.3 ml) in a sealed dry Hypovial containing a stirring bar. The addition took place at -60°C, with rapid stirring. The reaction mixture was stirred at this temperature for 30 min, then at room temperature for an additional 30 min. N^6-benzoyl-2'-deoxy-3'-O-levulinyladenosine **108** (500 mg, 1.10 mmol, coevaporated 3 times with anhydrous pyridine) was then added in DMF (5 ml), and the reaction allowed to stir at room temperature for 1 h. It was then poured into water and extracted with CH_2Cl_2. The organic layer was washed with water, dried (sodium sulfate) and evaporated to dryness. A silica gel column yielded the desired 3',5'-linked **104b** as a pure white foam (679 mg, .53 mmol, 47%) along with the expected symmetrically linked dimers. M. p. less than 90°, UV 280 nm, ^{1}H NMR ($CDCl_3$) 6.50 (m, 2, H1'), 3.72 (s, 6, -OCH_3), 2.15 (s, 3, -

$COCH_3$), 1.02 (bs, 14, -Si-$CH(CH_3)_2$), ^{29}Si NMR ($CDCl_3$) -7.06, Rf CH_2Cl_2:MeOH, 9:1, .49, solvent A, .10, EtOAc, .13. Mass spectral data, (triethanolamine matrix) m/z 1223.5, MH^+, rel. int. 28.13, m/z 984.3, MH^+ - Ad^{Bz}, rel. int. 17.20.

(b) N^6-benzoyl-5'-O-dimethoxytrityl-2'-deoxyadenosine **107** (1.7 g, 2.59 mmol) was dissolved in anhydrous DMF (20 ml). This solution was added dropwise over 30 min to a solution of diisopropyldichlorosilane (382 *ul*, 2.2 mmol) and imidazole (1.6 g, 8.8 mmol) in DMF (6 ml). The addition took place at -60°C with rapid stirring. The reaction mixture was kept at this temperature for 30 min after completion of the addition, then at room temperature for 30 min. N^6-benzoyl-2'-deoxy-3'levulinyladenosine **108** (582 mg, 1.29 mmol) was then added, as a solution in DMF (5 ml). This nucleoside had been coevaporated three times with anhydrous pyridine. The reaction mixture was allowed to stir at room temperature 1 h, then subjected to the usual workup and chromatography (CH_2Cl_2:solvent B, 1:0 to 0:1 gradient). The desired product **104b** was isolated in 60.5% yield (1.01 g, .78 mmol) as a white foam identical in all respects to that isolated in the previous paragraph. Also isolated was a mixture of the 3',3'-symmetrically linked dimer and a faster-moving material, chromatographically identical to the first addition product which is later consumed on addition of the second nucleoside. This is presumably the nucleoside silyl chloride, now hydrolysed to the nucleoside 3'-silanol.

Detritylation of 104b.

104b (928 mg, .74 mmol) was dissolved in trichloroacetic acid solution (3% w/v in CH_2Cl_2, 30 ml) and the bright orange solution was stirred 5 min at room temperature. After the reaction mixture was poured into aqueous sodium bicarbonate, the organic layer was separated, dried (Na_2SO_4) and concentrated. The solution was applied to a silica gel column (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient), which yielded the partially deprotected dimer **105b** as a white foam (574 mg, .58 mmol, 79% yield). M. p. 90-95°C, UV λmax 282 nm, ^{1}H NMR ($CDCL_3$) 6.50 (m, 1, H1'), 6.42 (m, 1, H1''), 2.20 (s, 3, -$COCH_3$), 1.08 (bs, 14, -Si-$CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .33, solvent A, .01, EtOAc, .00.

Synthesis of N^6-benzoyl-2'-deoxy-5'-O-dimethoxytrityl-3'-O-(5'-O-diisopropylsilyl-N^6-benzoyl-2'-deoxyadenylyl-3'-O-(5'-O-diisopropysilyl-N^6-benzoyl-2'-deoxy-3'-O-levulinyladenylyl))adenosine 109.

N^6-benzoyl-5'-dimethoxytrityl-2'-deoxyadenosine **107** (769 mg, 1.15 mmol) was dissolved in anhydrous DMF (10 ml) and added dropwise to a solution of diisopropyldichlorosilane (164 *ul*,

.94 mmol) and imidazole (718 mg, 10.5 mmol) in DMF (3 ml) kept at -60°C, with rapid stirring. The addition took 30 min. The reaction mixture was kept at low temperature for 30 min, then at ambient temperature another 30 min, before addition of the partially deprotected dimer **105b** (574 mg, .58 mmol, coevaporated 3 times with anhydrous pyridine) dissolved in DMF (5 ml). The reaction mixture was stirred at room temperature overnight (18 h) then worked up as usual. Silica gel column chromatography (CH_2Cl_2:solvent B, 1:0 to 0:1 gradient) yielded the desired fully protected trinucleotide analog **109** in 54% yield (540 mg white foam), along with the expected 3',3'-linked dimer and nucleoside silanol mixture described above. M. p. undefined (less than 90°C), UVλmax 282 nm, ^{1}H NMR ($CDCl_3$) 6.50 (m, 3, H1'), 3.73 (s, 6, -O-CH_3), 2.17 (s, 3, -$COCH_3$), 1.06 (m, ca. 28, -Si-CH(CH_3)$_2$), ^{29}Si NMR (CDCl3) -7.17, -7.69, Rf CH_2Cl_2:MeOH, 9:1, .39, solvent A, .01, EtOAc, .04. Mass spectral data (diethanolamine matrix) m/z 1690.8, MH^+.

Partial deprotections of the trinucleotide analog 109.

(a) Detritylation. **109** (275 mg, .18 mmol) was treated with trichloroacetic acid (3% w/v in CH_2Cl_2, 10 ml) for 5 min at room temperature. The usual workup and silica gel column (CH_2Cl_2:MeOH, 1:0 to 19:1 gradient) gave the detritylated trimer **110** as a white foam in 73% yield (131 mg). M. p. 88-91°C, UVλmax 278 nm, ^{1}H NMR ($CDCl_3$) 6.30-6.58 (bm, 3, H1'), 2.19 (s, 3, -$COCH_3$), 1.09 (m, 28, -Si-CH(CH_3)$_2$), Rf CH_2Cl_2:MeOH, 9:1, .35, solvent A, .00, EtOAc, .00.

(b) Delevulination. **109** (275 mg, .18 mmol) was treated with hydrazine solution (5 ml) for 20 min. The usual workup and chromatography (as above) gave the desired delevulinated compound **111** as a white glassy material (199 mg, 76% yield). M. p. 94-96°C, UV max 286 nm, NMR ($CDCl_3$) 8.80, 8.20 (m, 3, H2, H8), 6.44 (m, 3, H1'), 3.71 (s, 6, -OCH_3), 1.02 (m, 14, -Si-CH(CH_3)$_2$), Rf CH_2Cl_2:MeOH, 9:1, .36, solvent A, .00, EtOAc, .00.

Synthesis of the 2'-deoxyadenosine hexanucleotide analog 113.

111 (229 mg, .13 mmol) was dissolved in anhydrous DMF (5 ml) and added dropwise to a solution of diisopropyldichlorosilane (23 *u*l, .13 mmol) and imidazole (40 mg, .60 mmol) in DMF (1 ml). The addition took place over 30 min at -60°C, with rapid stirring. After the reaction mixture was allowed to stir at this temperature for 1 h, the cooling bath was removed and stirring continued 2 h at ambient temperature. **110** (200 mg, .13 mmol), dissolved in DMF (5 ml) was added and the reaction mixture stirred at room temperature overnight. The usual workup was followed by two columns. The first, run in a CH_2Cl_2 to CH_2Cl_2:MeOH 19:1 gradient, removed a

nontritylated material, which was the fastest spot on TLC. The second column, run in a CH_2Cl_2 to solvent A gradient, separated the two tritylated products. The faster of the two was identified as the 3',3'-linked hexamer by its ^1H NMR, which showed the absence of the levulinyl group, and the appropriate ratio of silyl to anomeric protons. The other tritylated material, isolated as 46 mg (11.5% yield) of a white foamy solid, was identified as the hexamer 113 by its ^1H NMR spectrum, especially the presence of the levulinyl and dimethoxytrityl signals, and the ratio of silyl to anomeric signals. M. p. not defined, UV λmax 270 nm, ^1H NMR ($CDCl_3$) 6.25-6.50 (bm, 6, H1'), 3.71 (s, 6, $-OCH_3$), 2.16 (s, 3, $-COCH_3$), 1.00 (bm, *ca.* 70-73, $-Si-CH(CH_3)_2$), Rf CH_2Cl_2:MeOH, 9:1, .44, solvent A, .00, EtOAc, .00.

The initial attempt at deprotection of this molecule was unsuccessful. Treatment of the hexanucleotide analog with TCA as described previously led to a single UV-absorbing product (other than dimethoxytritanol) whose chromatographic mobility was identical in several solvent systems to that of N^6-benzoyladenine. The ^1H NMR of this material was also identical to the protected free base. Thus the order of deprotection was altered. The hexamer (75 mg, .024 mmol, prepared as above) was delevulinated and debenzoylated by treatment with NH_4OH:MeOH (3:1, 4 ml) in 1,4-dioxane (5 ml) in a small, sealed flask for 6 h. The solvents were removed by lyopholisation. TLC (CH_2Cl_2:MeOH, 8:2) indicated a small amount of unreacted material. The treatment was repeated for an additional 6 h, followed again by lyopholisation. The product was purified on a small silica gel column (CH_2Cl_2:MeOH, 1:0 to 8.5:1.5 gradient) to yield 28 mg (49%) **114** (Rf CH_2Cl_2:MeOH, 9:1, .04, solvent A, .00, EtOAc, .00) of which 25 mg (.011 mmol) was immediately detritylated (TCA, 3% w/v in CH_2Cl_2, 5 ml) over 5 min. Column purification (CH_2Cl_2:MeOH, 1:0 to 8:2 gradient) yielded the fully deprotected product as a waxy solid (10 mg, 46%). UV λ max 260 nm, ^1H NMR (DMSO-d_6) 8.25, 8.24, 8.08, 8.07 (m, 6, H2, H8), 6.35 (m, 6, H1'), .95 (bs, *ca.* 70, $-Si-CH(CH_3)_2$).

Hypochromicity Studies.

For the hypochromicity studies, the UV absorbance of a 2 ml sample of the compound in water was measured. The sample was placed in a 5 ml polypropylene tube. The cell was washed twice with 1 ml of water and the washings were added to the tube. The water was removed by lyopholisation in the Speed-Vac^{tm}. NH_4OH:MeOH (4:1, 4 ml) was added to the tube and the tube was sealed with a rubber septum and tightly taped. After standing at 60°C overnight, the tube was placed in Dry Ice and then the solvents were lyopholised off. The residue was then redissolved in 2 ml water and the UV absorbance measured. Hypochromicity was calculated as

$$H=(A_{final}-A_{initial})/A_{final}$$

CD studies.

CD spectra were measured in water on a JASCO J-500C instrument in a 1 cm path length cell. For **100** and **114**, the instrument was set at a scan rate of 5 nm min^{-1}, and a sensitivity of .2 m^{o} cm^{-1} of the chart paper. For the natural nucleotides, a setting of 2 m^{o} cm^{-1} was used. The UV absorbance of the sample was measured immediately before the CD spectrum was measured. Molar ellipticity was calculated as

$$\Delta\epsilon = \theta^{o}/33 \times \text{molar concentration} \times \text{path length}$$

$$[\theta] = 3300 \times \Delta\epsilon$$

CONTRIBUTIONS TO KNOWLEDGE

A general procedure for the synthesis of arabino- and xylonucleosides was developed. This route is independent of the nature of the base (purine or pyrimidine) and uses the readily available nucleosides as the starting material, eliminating the necessity of coupling the base to the sugar. The reaction scheme is straightforward, and is compatible with protecting groups commonly encountered in nucleic acid chemistry.

A novel class of nucleotide analogues was developed In these molecules, silicon replaces phosphorus in the internucleotide link. It was shown that a variety of alkyl or aryl substituents may be used at silicon. The CD spectra of the silyl hexanucleotide analogues indicates that they may form a stable right-handed helix in water solution Synthesis and deprotection of these molecules is reasonably straightforward, although care should be exercised in the choice of non-nucleoside substituents at silicon Bulkier alkyl substituents such as isopropyl seem to provide the greatest stability The thymidine hexanucleotide analogue shows a hypochromicity close to that of natural hexathymidylic acid. Some suggestions for improving the analogues' solubility were made