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SYNTHESIS AND BINDING OF OLIGONUCLEOTIDES CONTAINING 2'-MODIFIED SULFIDE- OR SULFONE-LINKED DIMERS

by

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A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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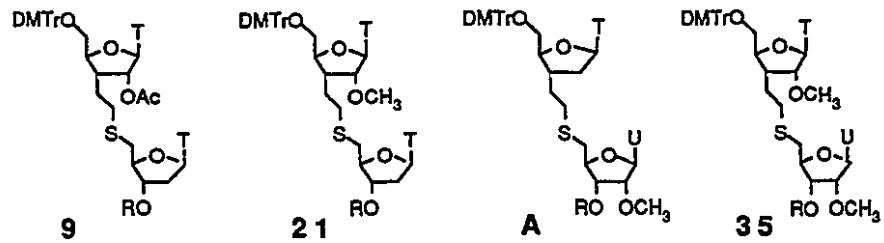
ABSTRACT

Three activated modified dimers **9**, **21** and **35**, which contain a dialkyl sulfide backbone, have been synthesized.

These dimers, as well as dimer **A**, have been incorporated into DNA strands by solid-phase techniques. The number of these dimers being incorporated varied from 1-3.



R = $iPr_2N(CNCH_2CH_2O)P$

Thermal studies have shown that the oligomers containing modified dimers indeed bind to their complementary DNA or RNA, except for two oligomers in which dimer **9** or **21** was incorporated three times. They only bind relatively poorly to complementary RNA, but not at all to DNA. The incorporation of **35** into DNA oligomers showed good binding to its complementary RNA, but not DNA.

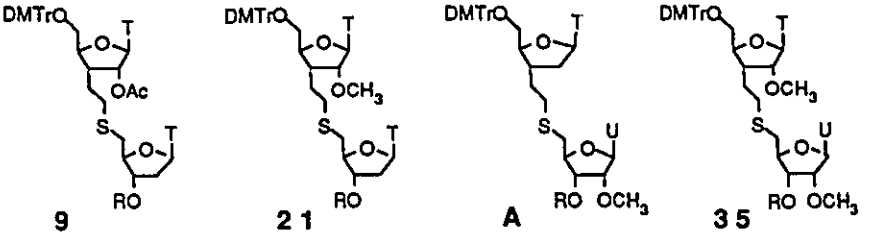
All sulfide-containing oligomers have been oxidized to sulfone-containing oligomers using oxone. In thermal studies, hybrids of the sulfone-containing oligomers with their complementary DNA and RNA showed much poorer binding properties than the corresponding sulfide-containing oligomers.

The synthesis of nucleoside **28**, the upper half of dimer **21**, as well as an improved procedure for the preparation of 2'-O-methyluridine, are described.

Résumé

La synthèse de trois dimères d'ADN modifiés (**9**, **21** et **35**) contenant un squelette dialkyle sulfure a été réalisée.

Ces dimères ont été incorporés dans une chaîne d'ADN en utilisant la synthèse en phase solide, le nombre de dimères incorporés par chaîne variant entre 1 et 3.



9 **21** **A** **35**

R = $iPr_2N(CNCH_2CH_2O)P$

Les études thermiques prouvent que les oligomères contenant ces dimères modifiés se lient à leur ADN ou ARN complémentaire, sauf pour deux oligomères contenant 3 dimères **9** et **21**. Ceux-ci se lient seulement faiblement à leur ARN complémentaire et pas du tout à l'ADN. L'incorporation de **35** donne des oligomères qui se lient bien à leur ARN complémentaire, et mal à leur ADN complémentaire.

Tous les oligomères contenant un squelette sulfuré ont été oxidés en oligomères contenant un lien sulfone, et leur abilité à se lier à leur ADN ou ARN complémentaire a été étudiée. Les études thermiques indiquent que les hybrides contenant un lien sulfone se lient beaucoup plus mal que ceux contenant un lien sulfure.

La synthèse du nucléoside **28**, la partie supérieure du dimère **21**, à partir d'un sucre est décrite, ainsi qu'une modification de la synthèse de la 2'-O-methyluridine.

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to Prof. George Just for his critical guidance and constant encouragement throughout my graduate years in his laboratory.

I also wish to thank:

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Everyone in Dr. Just's group for their help, especially Dr. Stephen H. Kawai and Dr. Daguang Wang for their cooperation and helpful discussion, Mr. Wenqiang Zhou for help in the operation of the DNA synthesizer, and Mr. Eric Marsault for translating the abstract into French.

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Dr. Orval A. Mamer for the measurement of mass spectra.

Dr. Françoise Sauriol for assistance in NMR experiments;

Ms. Renée Charron for help in administrative matters.

GLOSSARY OF SYMBOLS & ABBREVIATIONS

A	(deoxy)adenosine
Ac	acetyl (CH_3CO)
Bn	benzyl ($PhCH_2$)
br	broad (in NMR)
Bu	butyl (C_4H_9)
Bz	benzoyl (PhCO)
C	(deoxy)cytidine
calcd.	calculated
CAN	ceric ammonium nitrate
CI	chemical ionization
δ	chemical shift
d	doublet (in NMR)
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
disp.	dispersion
DMAP	4,4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMTr	4,4'-dimethoxytrityl
DMSO	dimethyl sulfoxide
DNA	2'-deoxyribonucleic acid
ds	double-stranded
Et	ethyl (C_2H_5)
equiv.	equivalent(s)
FAB	fast atom bombardment
g	gram(s)

G	(deoxy)guanosine (for N)
h	hour(s)
h	hextet (in NMR)
h^7	heptet (in NMR)
HIV	human immunodeficiency virus
HRMS	high-resolution mass spectrometry (spectrum)
Hz	Hertz
i-	*iso-*
l	wavelength
L	liter(s)
M	mole concentration
m	meter(s)
m	multiplet (in NMR)
m^n	symmetrical multiplet of n lines (in NMR)
min	minute(s)
m/e	mass-to-charge ratio
Me	methyl (CH_3)
mol	mole(s)
m.p.	melting point
mRNA	messenger RNA
MS	mass spectrometry (spectrum)
Ms	methanesulfonyl-
NMR	nuclear magnetic resonance
p-	*para-*
PAGE	polyacrylamide gel electrophoresis

Ph	phenyl (C_6H_5)
PNA	peptide nucleic acid
ppm	parts per million
Pr	propyl (C_3H_7)
PVP	poly-(4-vinylpyridine-co-styrene)
py	pyridine
q	quartet (in NMR)
res.	resolving power (in HRMS)
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
RT	room (ambient) temperature
s	singlet (in NMR)
ss	single-stranded
T	thymidine (for N)
t	triplet (in NMR)
t- or *tert-*	*tertiary-*
TBAF	tetra-*n*-butyl ammonium fluoride
TBDMSi	*tert*-butyldimethylsilyl
TBDPhSi	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
TIPDSi	1,3-dichloro-1,1,3,3,-tetraisopropyldisiloxane
t.l.c.	thin-layer chromatography
T_m	melting temperature
TMG	1,1,3,3-tetramethyguanidine
TMSi	trimethylsilyl-

TMSiOTf	trimethylsilyl triflate
TMS	tetramethylsilane
Tr	triphenylmethyl- or trityl-
tRNA	transfer RNA
U	uridine (for N)
UV	ultraviolet
v	volume
w	weight

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Chapter 1. Introduction

1.1 Nucleic Acid DNA and RNA

DNA and RNA contain three characteristic components: a nitrogenous base, a pentose, and a phosphoric acid bridge as the 3', 5'-linkage between each pentose unit. Five pyrimidine and purine bases are found in DNA and RNA which are cytosine (C), thymine (T), uracil (U), adenine (A) and guanine (G). The single important difference in the bases of DNA and RNA is that thymine is a principal base in DNA, but not often in RNA; conversely, uracil is a major one in RNA but rarely in DNA. The sugar moiety is 2'-deoxy-D-ribose in DNA, and D-ribose in RNA.

The structure of double-stranded (ds) DNA was proposed by Watson and Crick in 1954. It consists of two single DNA strands which form a right-handed double helix with base-pairing hydrogen bonds as recognition element. In this duplex, two strands are antiparallel, and the base-pairs consist of either A-T with two hydrogen bonds, or G-C with three hydrogen bonds. Single stranded DNA and RNA can also form other kinds of duplexes such as ds DNA : RNA and RNA : RNA (Figure 1).

Nucleic acids are a basic component of living cells. They play an important role in the processing of the growth, development and reproduction of living cells. DNA is the carrier of genetic information which is encoded by its sequence of bases. The genetic information in a gene is transmitted to progeny cells by the replication of DNA itself, and a gene DNA can be transcribed to yield complementary RNA, which, in turn, directs the synthesis of proteins. The self-replication of DNA is semiconservative. During the replication of DNA, a double helix is separated to two single strands and each strand is used as a template to

form a complementary daughter strand. In this way two new DNA duplexes identical to the parent DNA are synthesized.

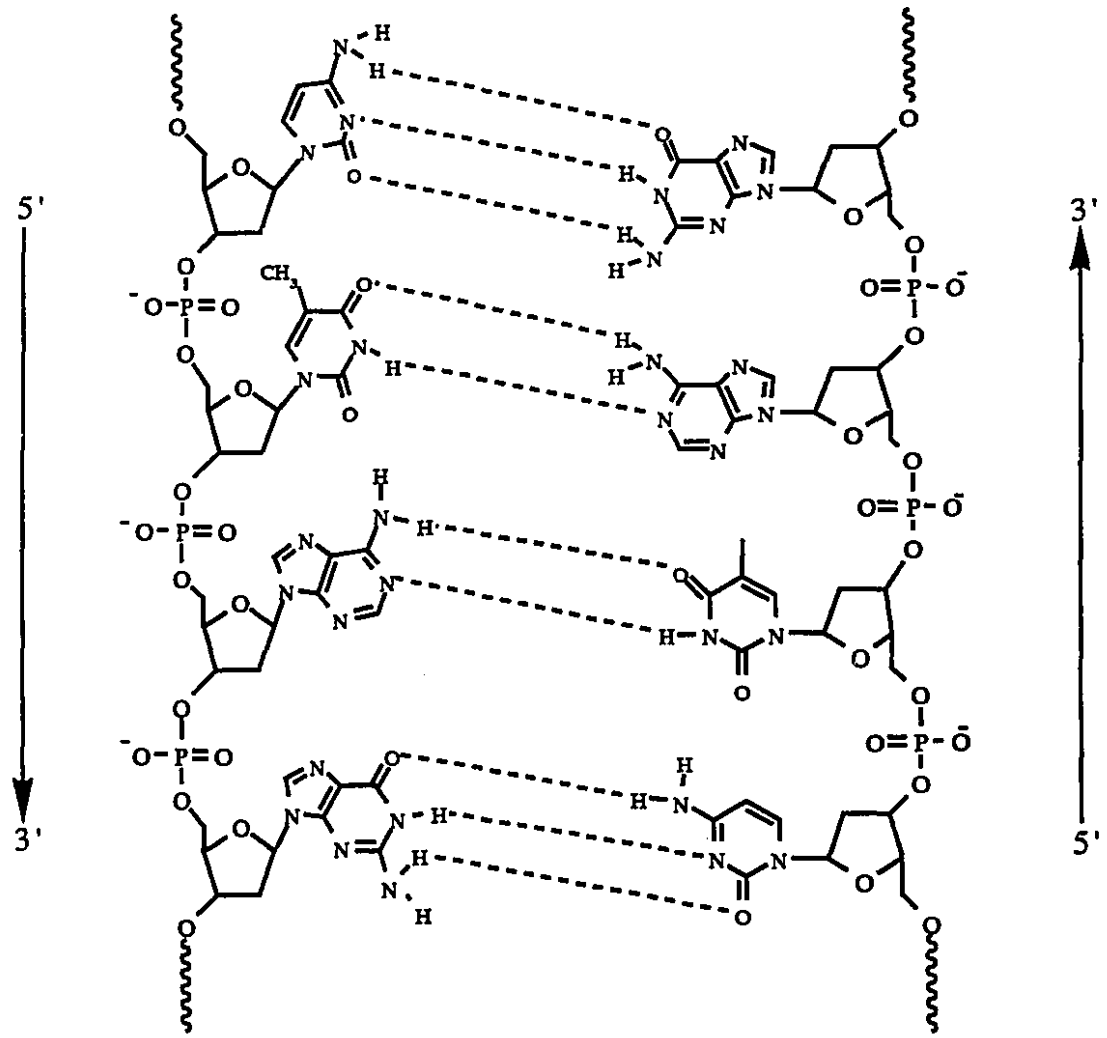

Figure 1 Watson-Crick base-pairing (The bases from top to bottom at the left side are C, T, A and G, at the right side G, A, T, C.).

The formation of RNA from a DNA sequence complementary to it constitutes the process of gene transcription. This transcription produces three kinds of RNA. They are messengers RNA (mRNA), transfer RNA (tRNA)

and ribosomal RNA (rRNA). mRNAs, as the term suggested, carries the genetic information transcribed from DNA to it. The function of mRNAs is to act as template to direct the synthesis of various proteins, and thus they can be a target for the regulation of protein synthesis. This regulation, when effected by formation of a double-stranded species consisting of mRNA (sense) and a complementary (antisense) strand, is referred to as the antisense strategy.

1.2 Antisense and Antigene Strategy

Oligonucleotides have the property to bind to their complementary strand. It is also known that certain oligonucleotides can bind to double-stranded DNA. Whereas, as was mentioned above, it is customary to refer to the the first process as the antisense strategy, and the second process is referred to as the antigene strategy because a double stranded DNA can be targeted.

Antisense

As shown in Figure 2, one gene may be transcribed to produce hundreds of mRNAs, each of which may then be translated to several hundred protein molecules. This translation process from mRNA to protein can be terminated by forming a duplex between an oligomer and mRNA[1].

Some natural antisense RNAs have been purified and their inhibiting function investigated[2]. They usually act as a modulator (inhibitor/stimulator) of RNA processing, as an inhibitor of transcription by causing premature termination of transcription, and as an inhibitor of translation by sequestering the ribosome binding site for a gene[3]. For example[4], in the bacterium *Escherichia coli,* the replication of plasmids is regulated by the interaction of target sense and antisense RNA.

Artificial antisense oligomers can also be employed in eukaryotic cells using the microinjection method. The first experiment was carried

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1 Winkler, M. E.; Mullis, K.; Barnett, J.; Stroynowski, I., Yanofsky, C.: *Proc. Natl. Acad. Sci. USA*, **79**, 2182 (1982).

2 Inouye, M.: *Gene*, **25**, 155 (1990).

3 Thoms, C. M.: *Antisense RNA and DNA*, Wiley-Liss, Inc. New York, 51 (1992).

4 Rosen, J.; Ryder, T.; Ohtsubo, H.; Ohtsubo, E: *Nature*, **290**, 794 (1981).

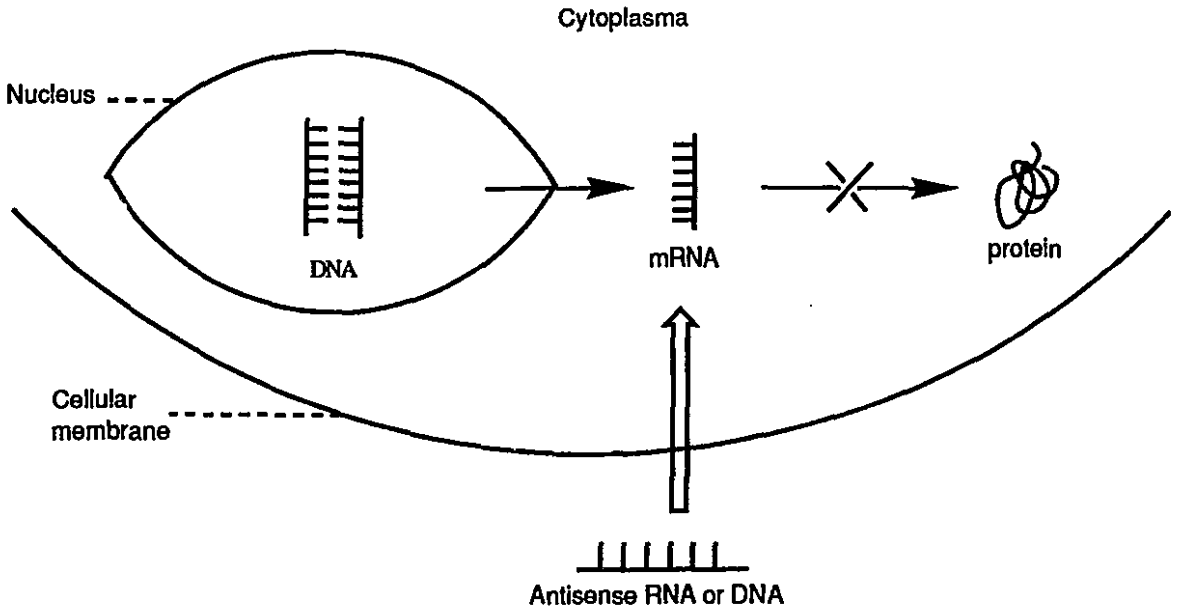

Figure 2 Mechanism of antisense DNA or RNA action.

out by Zamecnik and Stephenson in 1978[5]. Once the plasmid which can produce the related antisense RNA was injected into the tissue culture of a mouse cell containing a thymidine-kinase gene, the expression of this gene was reduced. Fütterer et al[6]. also showed that an antisense RNA can be used to inhibit the production of a deleterious DNA binding protein.

Clearly the application of the antisense technique is a very promising therapeutic strategy. . It means, for example, that the spread of a ssRNA virus can be effectively controlled. It is known that when a host cell is infected by a virus cell, the viral polymerase can catalyze the synthesis of a DNA complementary to the viral RNA, thus incorporating it into the host DNA. The production of these unwanted genes can be inhibited by the hybridization between viral RNA and an antisense oligonucleotide having the complementary sequence.

Today, automated DNA synthesizers make possible the synthesis of any desired DNA or RNA sequence[7]. The phosphorylated and protected nucleoside can be added sequentially to a growing DNA (or RNA) segment linked to a solid-support through a cycle of activation, coupling, capping, oxidation and detritylation (Figure 3). With the increasing length of an oligonucleotide the yield and purity of the synthetic oligomer is reduced; on the other hand, too short an antisense oligomer does not have the ability to bind selectively to the target mRNA and block the synthesis of proteins. A 17-18mer is a good choice, in that it is a unique sequence in the human genome, yet can be made in a relatively pure form.

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5 Zamecnik, P. C.; Stephenson, M. L.: *Proc. Natl. Acad. Sci. USA*, **75**, 280 (1978).

6 Fütterer, J.; Gordon, K.; Pfeiffer, P.; Hohn, T.: *Gene* , **67**, 141 (1988).

7 Beaucage, S. L.; Iyer, R. P.: *Tetrahedron* , **48**, 2223 (1992).

An oligonucleotide has to fulfil the following requirements:

1) It should be efficiently delivered into cells to achieve binding with cellular mRNA. It is believed that natural antisense oligonucleotides cannot cross lipid membranes of a cell because they are polyanionic molecules. Modified analogues may be nonionic and thus membrane-permeable.

2) Oligonucleotides should be resistant to nucleases present in cells and in the bloodstream.

3) Oligonucleotides must have sufficient affinity so that the complex formed with the target is stable enough to fulfil its functions. The melting temperature *Tm* of duplex is a measure to determine the specificity and affinity of an oligonucleotide to its target, and defines the temperature at which 50% the double strand has dissociated to two single strands.

4) Oligonucleotides must also be specific and bind only to their complementary sequence.

Although natural DNA and RNA can be readily synthesized and used as an antisense agent, they have major weaknesses when used as regulatory agents in a biological environment. In particular, they are not resistant towards nucleases, and they are hardly taken up by cells. In order to overcome those shortcomings, chemical modifications have been introduced into the molecules. For example, oligo(2'-O-alkylribonucleotides) in which the alkyl is cyanomethyl, methyl or allyl form very stable hybrids with complementary RNA sequences. Such modified oligonucleotides are useful antisense probes for investigating RNA and RNA protein complexes due to

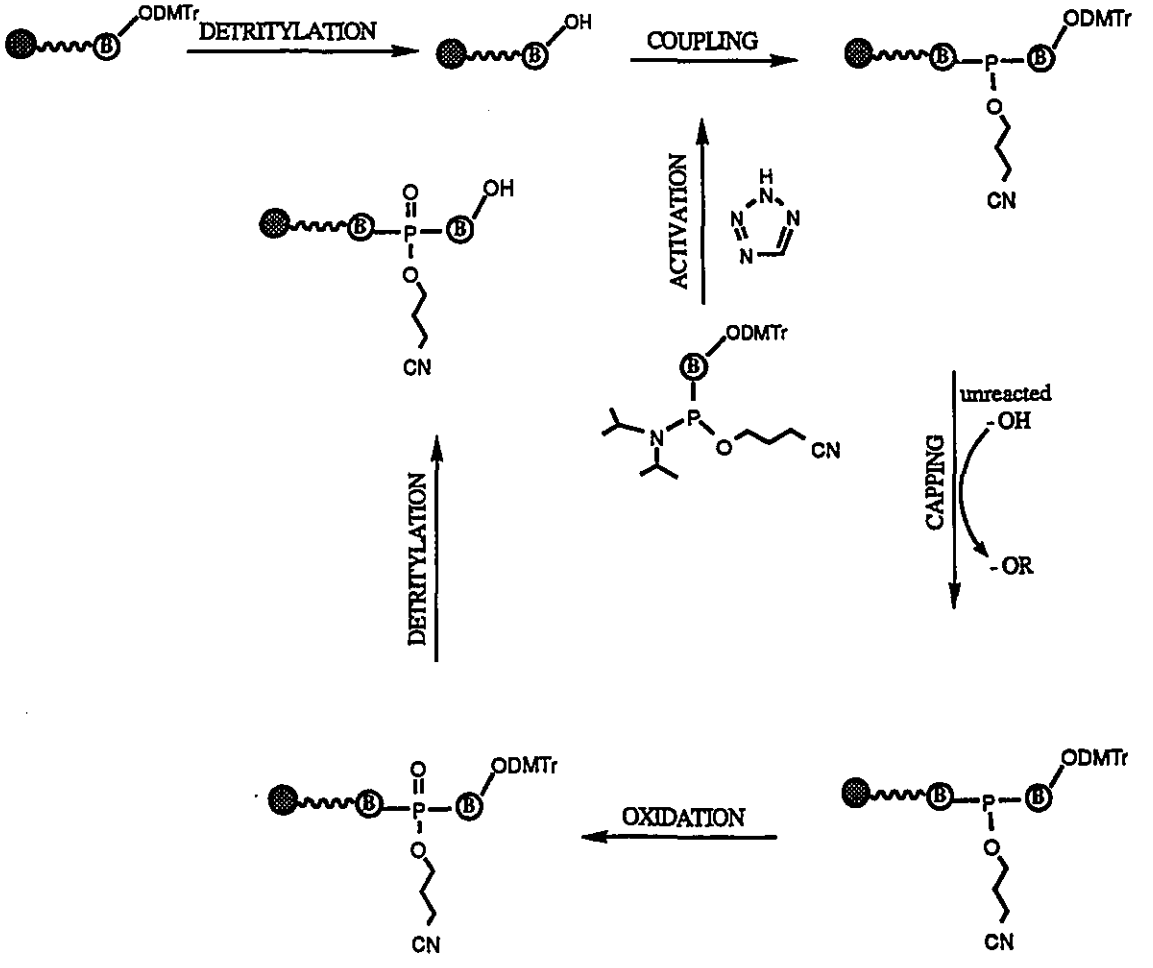

Figure 3 Phosphoramidite method for oligonucleotide synthesis on a solid support.

their resistance to degradation by RNA and DNA specific nucleases[8]. The details of such endeavors of modification will be introduced later.

Antigene Agents

It has been found that the functions of a gene can be inhibited by forming a triplex with the addition of a third DNA strand[9] (Figure 4). This strategy is called "antigene" by analogy with the "antisense" approach.

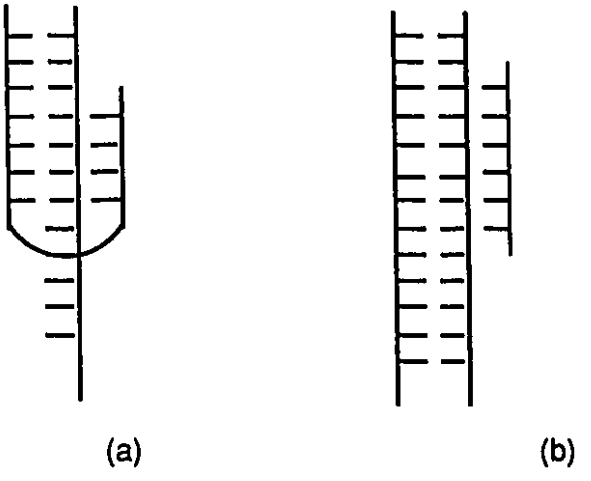

Figure 4 (a) intramolecular triplex; (b) external triplex.

In the structure of duplex DNA, base pairs are stacked and sugar phosphate backbones are wound around the helical axis, resulting in substantial differences in the relative width and depth of the major and minor grooves. An additional pyrimidine strand can specifically recognize

---

8 Sproat, B. S.; Beijer, B.; Douglas, M.; Garcia, R. G.; Lamond, A. I.; Paolella, G.: *Antisense Applications of Oligo(2'-O-Alkylribonucleotides); INSERM/NIH Conference*, Heidelberg, Germany (1992).

9 Morgan, A. R.; Wells, R. D.: *J. Mol. Biol.*, **37**, 63 (1968).

the purine strand of a Watson-Crick duplex DNA by Hoogsteen hydrogen bonded base triplets, TAT and C^+GT (Figure 5), the binding site of which is usually present in the major groove[10]. Thus, each purine base in a homopurine/homopyrimidine double helical DNA sequence offers two potential sequence-specific hydrogen bonds for oligonucleotide-directed triple helical recognition, and cytosine in the third strand must be protonated in order to form two hydrogen bonds with G in a G x C base pair.

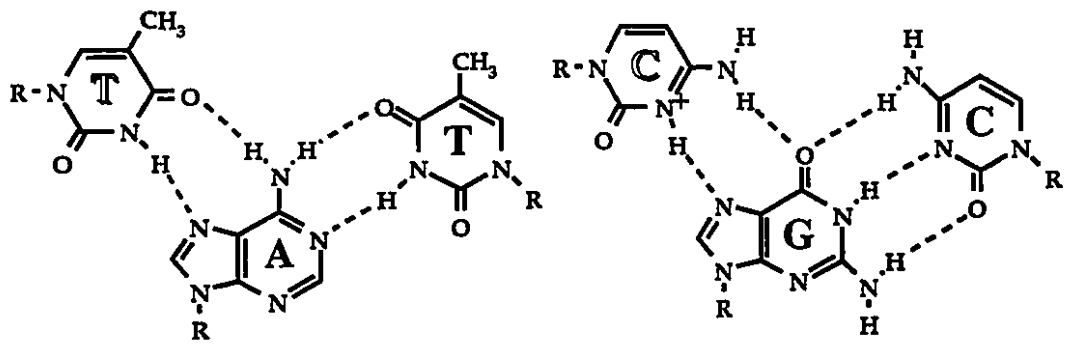

Figure 5 The pyrimidine oligonucleotide is bound by Hoogsteen hydrogen bonds in the major grooves to the purine strand in the Watson-Crick duplex.

Hogan and coworkers reported evidence for triplex formation at a single site with the human c-myc P1 promoter in *HeLa* cells[11]. In their experiments a synthetic 27-base-long oligodeoxyribonucleotide has been shown to bind to a duplex and thereby reduce c-myc mRNA levels. They

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10 Beal, P. A.; Dervan, P. B.: *J. Am. Chem. Soc.*, **114**, 4976 (1992).

11 Postel, E. H.; Flint, S. J.; Kessler, D. J.; Hogan, M. E: *Proc. Natl. Acad. Sci. USA*, **88**, 8227 (1991).

also reported that transcription of individual genes can be selectively modulated in living cells by sequence specific triplex formation[12].

12 Orson, F. M.; Thomas, D. W.; McShan, W. M.; Kessler, D. J.; Hogan, M. E.: *Nucl. Acids Res.*, **19**, 3435 (1991).

1.3 The First Generation of Modified Oligonucleotides

Modified phosphate linkages

Most of the early modifications were carried out on the phosphate linkage, since they could be done using a DNA-synthesizer in some modified form (Figure 6).

Phosphorothioates: Phosphorothioates, in which one nonbridging oxygen atom is replaced by a sulfur atom, is perhaps the earliest used and most studied class of antisense nucleic acid analogues. Such analogues exhibit an enhanced binding ability with their complementary RNA and anti-HIV activity in *vitro*[13]. They also show greater extra- and intracellular longevity because of increased nuclease resistance[14]. It is of great interest that the duplex of a phosphorothioate and RNA is susceptible to cleavage by RNaseH[15]. However, the introduction of a sulfur atom (or other groups) makes the phosphorus atom chiral and thus results in a mixture of 2^n diastereomers for a (n+1)-mer (Figure 7). In addition, with the negative charge on sulfur, phosphorothioates do not penetrate cells efficiently. Some phosphorothioates are at present undergoing clinical trial[16].

Methylphosphonates: One of the efforts to eliminate the negative charge on the internucleotide linkage is the formation of

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13 Matsukura, M.; Shinozuka, K.; Zon, G.; Mitsuya, H.; Reitz, M.; Cohen, J. S.; Broder, S.: *Proc. Natl. Acad. Sci. USA*, **84**, 7706 (1987).

14 Matsukura, M.: *Antisense RNA and DNA*, Wiley-Liss, NY, 285 (1992).

15 Furdon, P. J.; Dominski, Z.; Kole, R.: *Nucl. Acids Res.*, **17**, 9193 (1989).

16 Cook, P. D., ISIS Pharmaceuticals, personal communication.

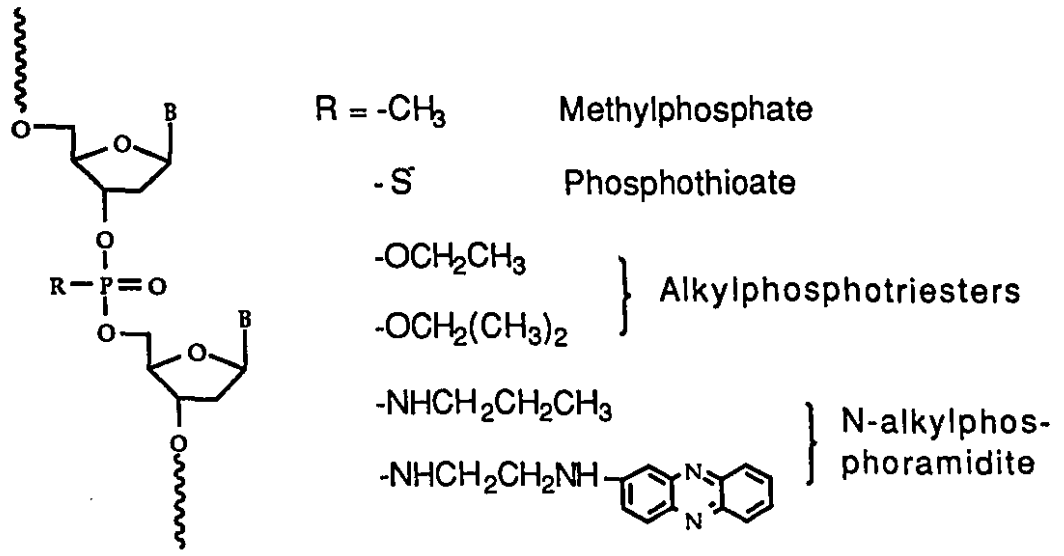

Figure 6 Modification of internucleotide phosphate bond.

methylphosphonates [17,18,19]. The oligodeoxynucleotides containing 3', 5' linked methylphosphonate internucleotide bonds are readily synthesized on solid-phase supports. These oligomers are resistant to nuclease hydrolysis, can pass through the membranes of mammalian cells in culture and can form stable duplexes with complementary RNAs. An 8-mer incorporating methyloligodeoxynucleotides can be targeted to the donor or acceptor splice junctions of virus pre-RNA and thus selectively inhibit virus protein synthesis and growth. Work in this area is pursued intensively and this class of compounds has therapeutic potential.

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17 Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'o, P. O. P.: *Biochemistry*, **18**, 5134 (1979).

18 Miller, P. S.; Agris, C. H.; Aurelian, L.; Blake, K. R.; Murakami, A.; Reddy, M. P.; Spitz, S. A; Ts'o, P. O. P.: *Biochimie*, **67**, 769 (1985).

19 Ts'o, P. O. P.; Miller, P. S.; Aurelian, L.; Murakami, A.; Agris, C.; Blake, K. R.; Lin, S-B.; Lee, B. L.; Smith, C. C.: *Ann. N. Y. Acad. Sci.*, **507**, 220 (1988).

Phosphotriesters: Unlike methyl or cyanoethyl groups which are used to protect the phosphates and are easily removed in the coupling reaction of two nucleosides, ethyl[20] and isopropyl[21] phosphate triesters are stable during the cleavage from the support and the deprotection of the bases, which is carried out with 25% NH_4OH at room temperature for 48 hour[22]. Those phosphotriesters are less effective inhibitors compared to phosphorothioates in the inhibition of chloramphenicol acetyl transferase gene expression[23].

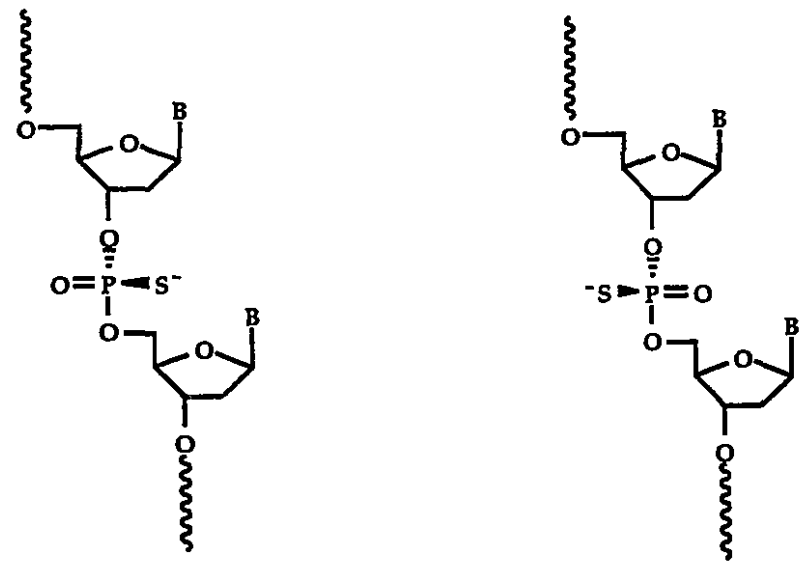

Figure 7 Chirality of phosphorus.

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20 Gallo, K. A.; Shao, K-L.; Phillips, L. R.; Regan, J. B.; Koziolkiewicz, M.; Uznanski, B.; Stec, W. J.; Zon, G.: *Nucl. Acids Res.*, **14**, 7405 (1986).

21 Stec, W. J.; Zon, G.; Gallo, K. A.; Byrd, R. A.; Uznanski, B.; Guga, P.: *Tetrahedron Lett.*, **26**, 2191 (1985).

22 Guga, P.; Koziolkiewicz, M.; Obruszek, A.; Uznanski, B.; Stec, W. J.: *Nucleosides Nucleotides*, **6**, 111 (1987).

23 Marcus-Sekura, C. J.; Woerner, A. M.; Shinozuka, K.; Zon, G.; Quinnan, Jr, G. V.: *Nucl. Acids Res.*, **15**, 5749 (1987).

Phosphoramidites: Phosphoramidites have also been prepared, and a few reports dealt with their usage as antisense reagents[24].

Some laboratories focused on the introduction of functional groups at the 5'- or 3'-ends of oligonucleotides. Thus, 5'-cholesteryl[25], 5'-*N*-alkylphosphoramidyl[26], and 3'-sulfhydryl[27] groups and vitamin E attached to the 3'- or 5'-position[28] were introduced. The modifications at the end of the oligomers can protect the rest of the molecule from nucleases and increase membrane penetration.

Modified Sugars

RNA-RNA duplexes are generally more stable than DNA-DNA or DNA-RNA[29] duplexes, indicating that a simple modification at the 2'-position has a large effect on duplex stability.

One of the early modification of the sugar was the methylation of the 2'-hydroxyl group[30]. The methyl substitution at the 2'-OH function results in a modified oligoribonucleotide with improved resistance to nuclease degradation[31] compared to that of natural RNAs, which are inherently unstable under physiological conditions. The thermal stability

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24 Jäger, A.; Levy, M. J.; Hecht, S. M.: *Biochemistry.*, **27**, 7237 (1988).

25 Letsinger, R. L.; Zhang, G.; Sun, D. K.; Ikeuchi, T.; Sarin, P. S.: Proc. *Natl. Acad. Sci USA*, **86**, 6553 (1989).

26 Schubert, F.; Ahlert, K.; Cech, D.; Rosenthal, A.: *Nucl. Acids Res.*, **18**, 3427 (1990).

27 Gupta, K. C.; Sharma, P.; Kumar, P.; Sathyanarayana, S.: *Nucl. Acids Res.*, **19**, 3019 (1991).

28 Will, D. W.; Brown, T.: *Tetrahedron Lett.*, **33**, 2729 (1992).

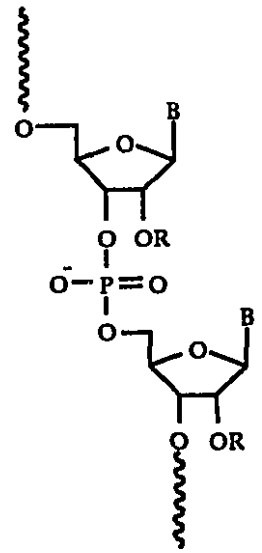
29 Uhlmann, E.; Peyman, A.: *Chem. Rev.*, **90**, 543 (1990).

30 Mukai, S.; Shibahara, S.; Morisawa, H.: *Nucl. Acids Res. Symp. Ser.*, **19**, 117 (1989).

31 Sproat, B. S.; Lamond, A. I.; Beijer, B.; Neuner, P.; Ryder, U.: *Nucl. Acids Res.*, **17**, 3373 (1989).

of a 2'-O-methylribonucleotide : RNA duplex is found to be greater than that of the complementary RNA-RNA or DNA-DNA duplexes[32].

Recently, a series of 2'-O-alkylated oligoribonucleotides have been synthesized and utilized as antisense probes (Figure 8). They include allyl[33] and 3,3-dimethylallyl[34], ethyl, propyl, and other substituents[35]. The thermal stability of their duplexes is increased with the size of the substituents.



R = $-CH_3$,
$-CH_2-CH=CH_2$ (allyl),
$-CH_2-CH=C(CH_3)_2$ (3,3-dimethylallyl),
$-CH_2-CH_3$ (ethyl),
$-(CH_2)_2-CH_3$ (propyl),
$-(CH_2)_3-CH_3$ (butyl),
$-(CH_2)_4-CH_3$ (pentyl),
$-(CH_2)_8-CH_3$ (nonyl).

Figure 8 2'-O-Alkylated sugar.

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32 Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E.: *Nucl. Acids Res.*, **15**, 6131 (1987).

33 Sproat, B. S.; Iribarren, A. M.; Garcia, R. G.; Beijer, B.: *Nucl. Acids Res.*, **19**, 733 (1991).

34 Iribarren, A. M.; Sproat, B. S.; Neuner, P.; Sulston, I.; Ryder, U.; Lamond, A. I.: *Proc. Natl. Acad. Sci. USA*, **87**, 7747 (1990).

35 Lesnik, E. A.; Guinosso, C. J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L. L.; Ecker, D. J.; Cook, P. D.; Freier, S. M.: *Biochemistry*, **32**, 7823 (1993).

2'-Deoxy-2'-fluoroadenosine, -guanosine, -uridine, and -cytidine have been successfully incorporated into phosphorothioates[36]. The stabilizing effects of the 2'-deoxy-2'-fluoro modifications on RNA-DNA duplexes are shown to be superior to those of the 2'-O-methyl substitutions. Modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides also afforded antisense molecules with improved stability toward nucleases.

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36 Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzalez, C.; Cook, P. D.: *J. Med. Chem.*, **36**, 831 (1993).

1.4 Modified Oligonucleotides with Dephospho Backbones

As described above, modified oligonucleotides, where a functional group replaces one of the nonbridging oxygens in the internucleotide bond, show enhanced cellular uptake and increased nuclease resistance. Unfortunately, the replacement generates chirality because four different groups are attached to the phosphorus atom. Since the synthesis is not stereospecific, each modified oligonucleotide will be a heterogeneous mixtures. In addition, less active or inactive forms may dilute the potency of the active one. An alternate modification method to obtain achiral oligonucleotides is to replace the phosphodiester bridge by an entirely different group (Figure 9)[37].

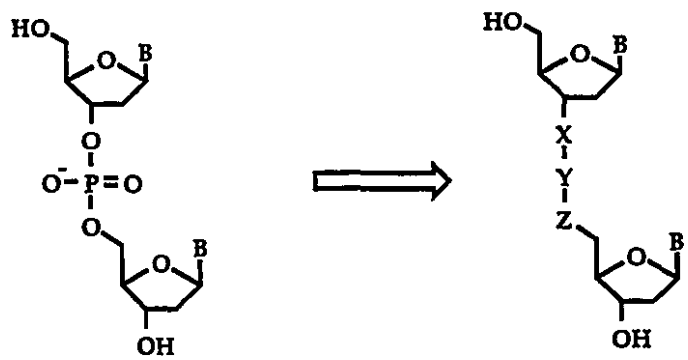

Figure 9 Site of modification.

Carbamate linkages replacing the phosphate bond are a widely studied substitution. Different synthetic routes for the preparation of the oligonucleotides bearing the carbamate linkage have been reported by

37 Sanghvi, Y. S.; Cook, P. D.: *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*, Plenum Publishing Co. NY, inpress (1993).

several authors[38,39,40,41]. The carbamate dimer was stable towards hydrolysis by nucleases. A modified hexamer C*C*C*C*C*C (* represents a carbamate-linkage) exhibited an unusually high binding *Tm* (40°C higher than control)[41], whereas a thymidine hexamer linked by carbamate bridge failed to show any hypochromicity[40].

Dodecamers which incorporated amide- or N-methyl amide-linked thymidine dimers have been synthesized in our laboratory, and elsewhere[42]. This class of modifications demonstrates the advantage that it is nonionic, much less polar than the phosphate, and can be made even more lipophilic by *N*-alkylation. Thermal denaturation studies indicated that these modifications caused little or no destabilization of the DNA/RNA duplex.

In order to circumvent the chirality problem, a novel methylhydroxyamine-linked dimer was incorporated into a 16mer DNA[43]. Hybridization studies indicated that the uniform distribution of modified dimers provided a more stable modified-DNA/RNA duplex than the control DNA/RNA duplex. These modified oligomers also showed significant resistance to nucleases when incubated with *HeLa* cellular extracts.

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38 Gait, M. J.; Jones, A. S.; Walker, R. T.: *J. Chem. Soc. Perkin Trans.*, **1**, 1684 (1974).

39 Mungall, W. S.; Kaiser, J. K.: *J. Org. Chem.*, **42**, 703 (1977).

40 Coull, J. M.; Carlson, D. V.; Weith, H. L.: *Tetrahedron Lett.*, **28**, 745 (1987).

41 Stirchak, E. P.; Summerton, J. E.: *J. Org. Chem.*, **52**, 4202 (1987).

42 (a) Idziak, I.; Just, G., Damha, M. J., Giannaris, P. A.: *Tetrahedron Lett.*, **34**, 5417 (1993); (b) Lebreton, J.; Waldner, A.; Lesueur, C.; Mesmaeker, A. D.: Synlett, 137 (1994).

43 Vasseur, J-J; Debart, F.; Sanghvi, Y. S.; Cook, P. D.: *J. Am. Chem. Soc.*, **114**, 4006 (1992).

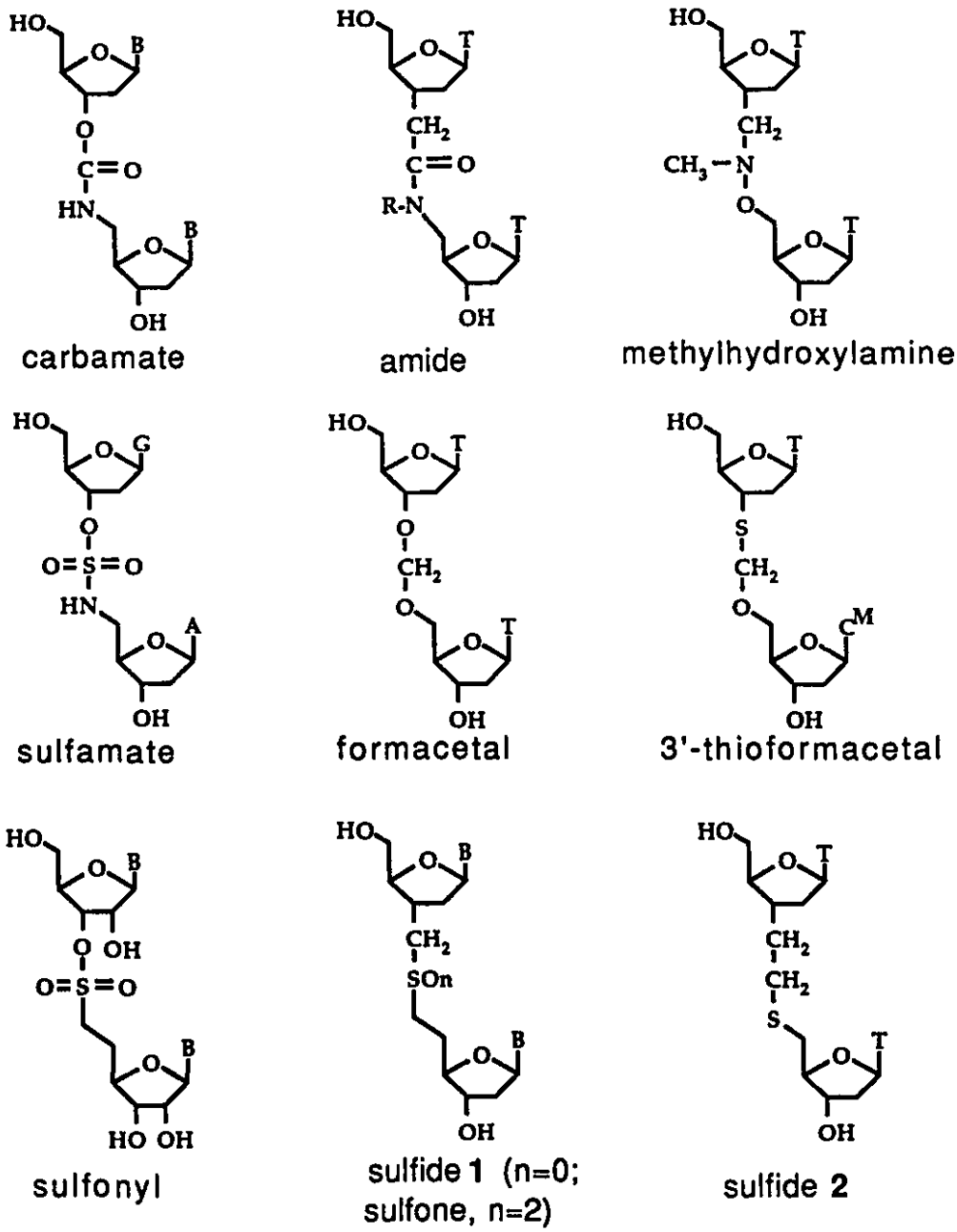

Figure 10 Oligonucleotides with dephospho backbone.

The preparation of the oligonucleotides in which one phosphate was replaced by a sulfamate has been done by Huie *et al*[44]. The sulfamate-linkage was positioned between the residues where *EcoR1* is normally cleaved. The sulfamate modification was found to be fully resistant to this enzyme, while the phosphodiester linkage were cleaved quantitatively. Its introduction resulted in a decrease of the *Tm* by 3°C.

As a simple, small and achiral isostere for the phosphate linkage, the formacetal linkage is attractive to chemists[45]. An altered 14-mer with three formacetal-modified dimers had a *Tm* very close to its parent DNA/RNA. The interesting point was when it was hybridized with complementary DNA, there was a remarkable drop in *Tm* as compared to the parent DNA/DNA. This dimer was also stable in the presence of snake venom phosphodiesterase.

Replacement of 5'-oxygen of formacetal with a sulfur has been reported[46]. The binding properties of this analog were poor. But, when the replacement occurred at 3'-oxygen, the 3'-thioformacetal/RNA duplex gave a 5.5°C enhancement in *Tm* relative to the control one[47]. This improvement was attributed to a lesser steric interaction of 3'-sulfur than that of its 5'-isomer.

The synthesis of sulfonyl-containing ribodinucleosides has been described by Musicki and Widlanski[48]. These sulfonyl-containing dimers are stable under basic conditions.

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44 Huie, E. M.; Kirshenbaum, M. R.; Trainor, G. L.: *J. Org. Chem.*, **57**, 4569 (1992).

45 Matteucci, M.: *Tetrahedron Lett.*, **31**, 2385 (1990).

46 Matteucci, M.; Lin, K-Y.; Butcher, S.; Moulds, C.: *J. Am. Chem. Soc.*, **113**, 7767 (1991).

47 Jones, R. J.; Lin, K-Y.; Milligan, J. F.; Wadwani, S.; Matteucci, M. D.: *J. Org. Chem.*, **58**, 2983 (1993).

48 Musicki, B.; Widlanski, T. S.: *Tetrahedron Lett.*, **32**, 1267 (1991).

The synthesis of a series of nucleotide analogues containing sulfur or sulfone backbone has also been described. The sulfide (sulfone) **1** was synthesized the first time in 1990[49]. Recently, an improved method to obtain this analogue was published[50]. However, no data about its binding or enzyme study has been reported. A full synthetic route of thymidine building blocks for sulfide **2** has been published by our group[51]. This dimer was incorporated three times into a 12-mer DNA and the binding properties were investigated[52]. This system will be further discussed later.

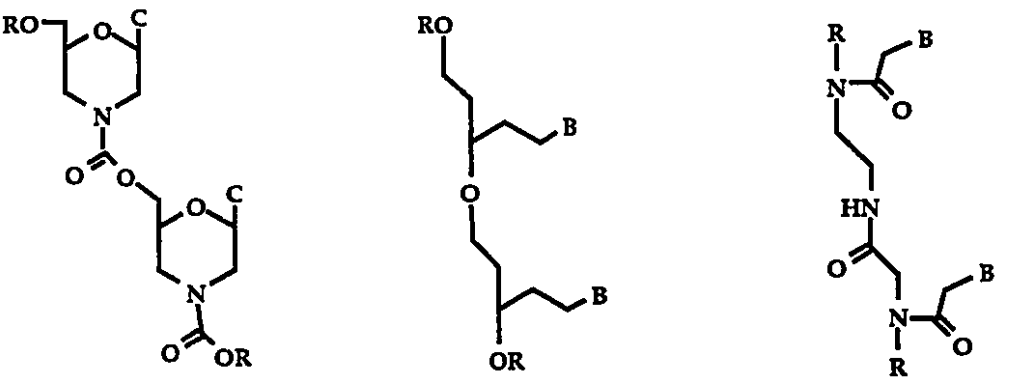

Figure 11 Unnatural nucleoside units.

Besides these dephospho modifications described above, there were a few examples of unnatural nucleoside-like building blocks (Figure 11).

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49 Schneider, K. C.; Benner, S. A: *Tetrahedron Lett.*, **31**, 335 (1990).

50 Huang, Z.; Benner, S. A.: *Synlett.*, 83 (1993).

51 Kawai, S. H.; Wang, D.; Just, G.: *Can. J. Chem.*, **70**, 1573 (1992).

52 Kawai, S. H.; Wang, D.; Giannaris, P. A.; Damha. M. J.; Just, G.: *Nucl. Acids Res.*, **21**, 1473 (1993).

Morpholine subunits linked by carbamate groups offered greater thermal stability relative to the DNA/DNA control, while it did not recognize complementary RNA[53].

Oligonucleotides containing acyclic "carbohydrate" components such as showed in Figure 11, demonstrated remarkable resistance against enzymatic breakdown, but the duplexes formed between these oligomers and their complementary DNA showed a much decreased thermal stability[54].

Peptide nucleic acid (PNA) is another new system which allows the facile design of an achiral backbone and relatively large-scale production. These studies showed that PNA-DNA had much higher *Tm* values than that of the corresponding DNA/DNA, and were stable to enzyme degradation[55].

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53 Stirchak, E. P.; Summerton, J. E.; Weller, D. D.: *Nucl. Acids Res.*, **17**, 6129 (1989).

54 Vandendriessche, F.; Augustyns, K.; Aerschot, A. V.; Busson, R.; Hoogmartens. J.; Herdewijn, P.: *Tetrahedron*, **49**, 7223 (1993).

55 Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H.: *J. Am. Chem. Soc.*, **114**, 1895 (1992).

1.5 Triplex Formation

The DNA double helix is the most important component in living systems. In it is encoded the genetic information, which establishes all the fundamental functions of life. One of the goals of medicinal chemistry is to inhibit the expression of unwanted genes. Triple helix formation may be a technique to achieve this purpose. A large amount of work has been devoted to the investigation of triple helix formation[56].

There are a few instances where the antigene strategy has been implicated. Strobel et al[57] showed that oligonucleotides with 15 pyrimidine bases in length, equipped with a cleaving function (EDTA·Fe) at the 5'-end, caused sequence specific double strand breakage at one site in plasmid DNA which was 4.0 kilobase pairs in size.

Another example of the triplex formation at neutral pH was described by Ono et al[58]. 2'-O-methylpseudoisocytidine was synthesized and incorporated into a pyrimidine DNA. This kind of modified oligomers can form a triplex with a duplex DNA at pH 7.2. Under the same conditions no triplex formation could be detected with oligomers containing 5-methyl-2'-deoxycytidine.

Similarly, triple helix binding was improved using 5-methylcytosine instead of cytosine in the third pyrimidine strand[59], since this cytosine is protonated at pH 6.7 rather than pH 5.7 for the unsubstituted cytosine.

Oligonucleotide (14-mer) containing modified formacetal dimers were one of the earliest dimers which incorporated "dephospho" backbone

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56 Thuong, N. T.; Helénè, C.: *Angew. Chem. Int. Ed. Engl.*, **32**, 666 (1993).

57 Strobel, S. A.; Moser, H. E.; Dervan, P. B.: *J. Am. Chem. Soc.*, **110**, 7927 (1988).

58 Ono, A.; Ts'o, P. O. P.; Kan, L.: *J. Org. Chem.*, **57**, 3225 (1992).

59 Xodo, L. E.; Manzini, G.; Quadrifoglio, F.; van der Marel, G. A.; van Boom, J. H.: *Nucl. Acids Res.*, **19**, 5625 (1992).

dimers and were reported to have the ability to form a triplex with duplex DNA under physiological conditions[47]. The DNA footprinting results showed that neutral achiral formacetal analogues were capable to form sequence-specific triplex at pH 6.8.

Recently, Jones et al reported that they had synthesized riboacetal dimers and incorporated them into 15-mer as thymine-thymine and thymine-5-methylcytosine dimer blocks[60]. These oligomers containing riboacetal dimers exhibited enhanced affinity for double strand DNA compared to the phosphodiester control.

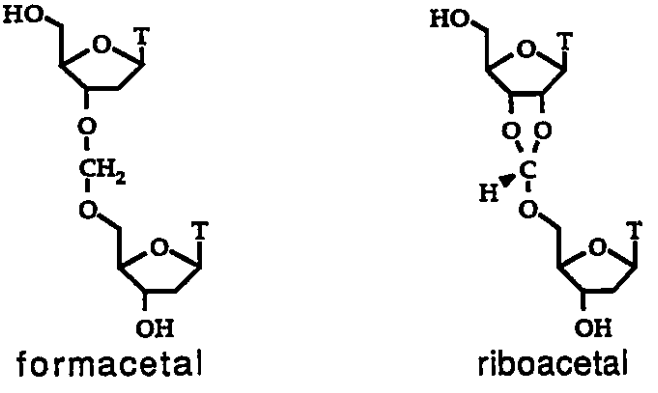


Figure 12 Modified dimers.

60 Jones, R. J.; Swaminathan, S.; Milligan, J. F.; Wadwani, S.; Froehler, B. C.; Matteucci, M.: *J. Am. Chem. Soc.*, **115**, 9816 (1993).

1.6 Plan of Study

For treatment of diseases on the DNA or RNA level, unmodified oligonucleotides cannot be used since they are not resistant to nuclease degradation and are not taken up by cells. Although modifications of the phosphate-linkage increase stability towards enzyme hydrolysis and membrane penetration, they possess a stereocenter and produce heterogeneous mixtures. Therefore, the plan of this study focuses on the design and synthesis of oligonucleotides in which the phosphate-linkage is totally replaced.

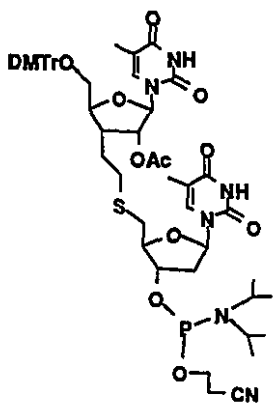
These modified analogues (1) must be chemically synthesizable and stable; (2) should not have stereocenter in the molecule; (3) should be non-ionic so that they are able to penetrate membrane. A thioether-linked system in which the 3'- and 5'-carbons of adjacent sugars are joined by an alkane chain containing a single sulfur atom could satisfy the above conditions.

Since RNA-RNA duplexes are more stable than DNA-DNA duplexes, and the predominant *in vivo* target of antisense oligonucleotides is RNA, the first target molecule to be synthesized will be a mixed ribo-deoxyribo dinucleoside analogue (Scheme 1a), which carries a hydroxyl group at the 2'-position of one sugar and where the two sugars are linked by an alkyl thioether.

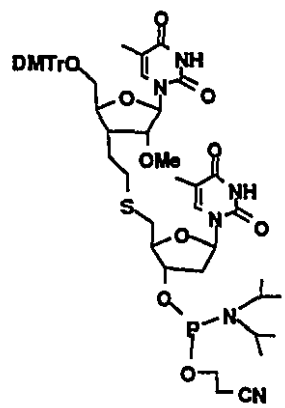
Considering that methylation at 2'-OH position of unmodified oligonucleotides gave enhanced binding results corresponding to their parent ones, two methylated sulfide dimers will be synthesized (Scheme 1b and 1c).

These dimers will be incorporated into DNA sequences, individually, in order to measure their affinity to complementary strands.

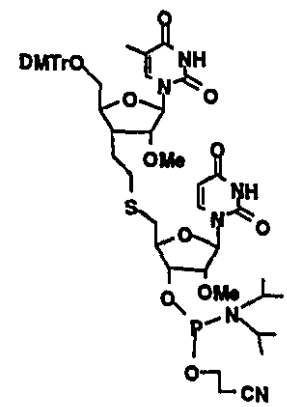
Scheme 1



a



b

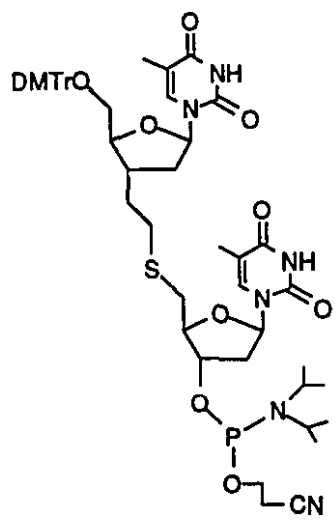


c

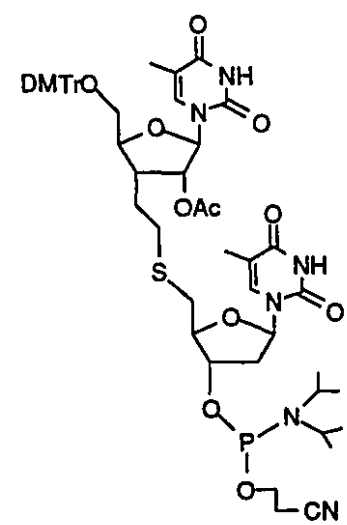
Chapter 2. Synthesis of Sulfide-linked Dimer (9)

In work previously carried out in our laboratory, DNA strands incorporating an internucleosidic diakyl sulfide **S1** (Scheme 2) were synthesized and shown not to be degraded by nucleases. These sulfide-containing strands also exhibited cooperative binding to both fully natural DNA and RNA[52] although the *Tm* values were lower than that of DNA/DNA or DNA/RNA hybrids. In order to better understand the properties of the oligomers containing modified dimers, in which phosphodiester-linkage is totally replaced by dialkyl sulfide, the preparation of a mixed ribo-deoxyribo dinucleoside analogue (hydroxy sulfide) **9** was chosen as the first target.

Scheme 2



S1

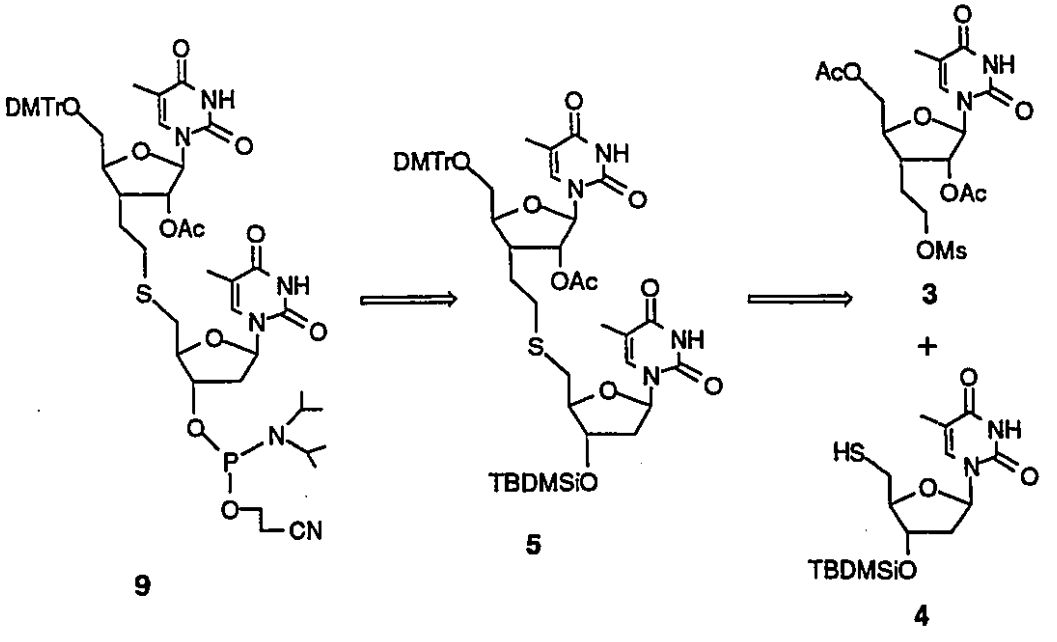


9

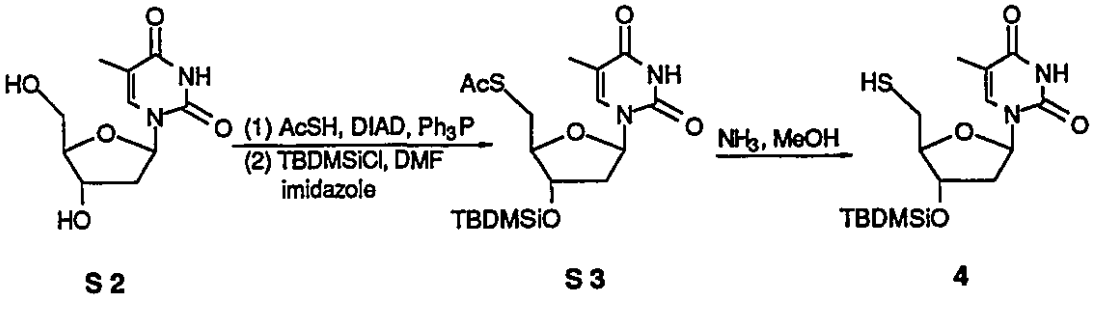
The retrosynthesis of dinucleoside **9** is shown in Scheme 3. Dimer **5** is the key intermediate in the synthesis of compound **9**. It is obtained by the coupling reaction of mesylate **3** and thiol **4**. The preparation of thiol **4**

was performed on a large scale from thymidine by the previously described[51] thiolation and deacetylation (Scheme 4).

Scheme 3



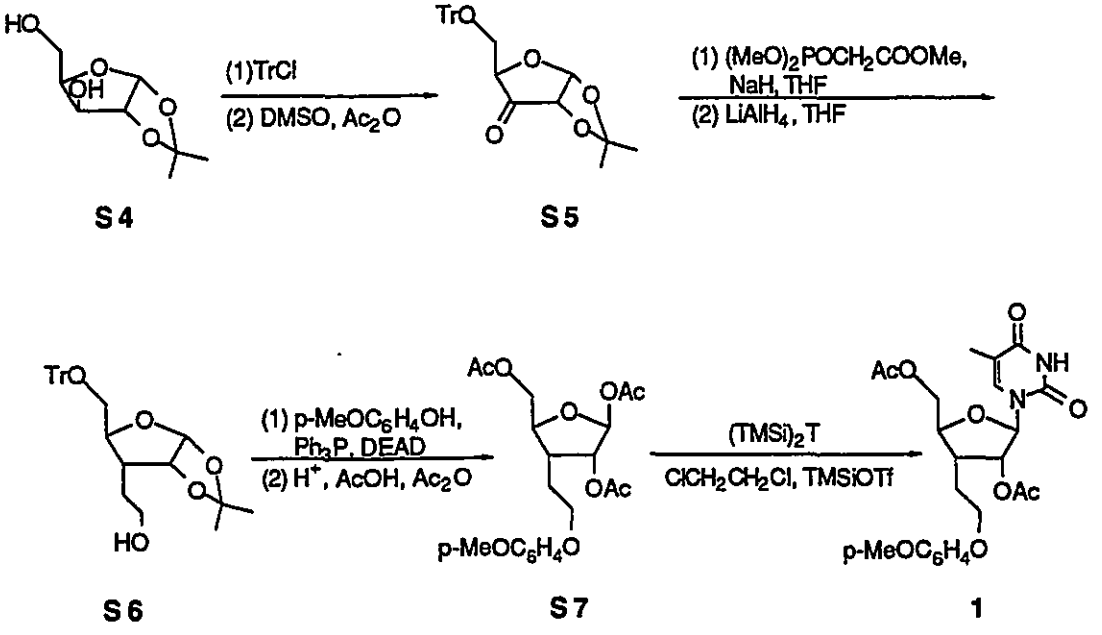
Scheme 4



2.1 Synthesis of Alcohol (2) and Mesylate (3)

The starting material, 3'-deoxy-3'-(2-hydroxyethyl)ribofuranosylthymine derivative **1**, was obtained according to literature procedures, and is outlined in Scheme 5[51,61,62].

Scheme 5



The oxidative removal of the *p*-methoxyphenyl group was effected by treatment with ceric ammonium nitrate (CAN) in an acetonitrile-water solution at 0°C to give alcohol **2** in 76% yield (Scheme 6). The pH of the reaction solution was around 4. In this case, the acetate groups at 2'- and 5'-positions were very stable to the reaction conditions. Slow addition of methanesulfonyl chloride to the solution containing alcohol **2** and

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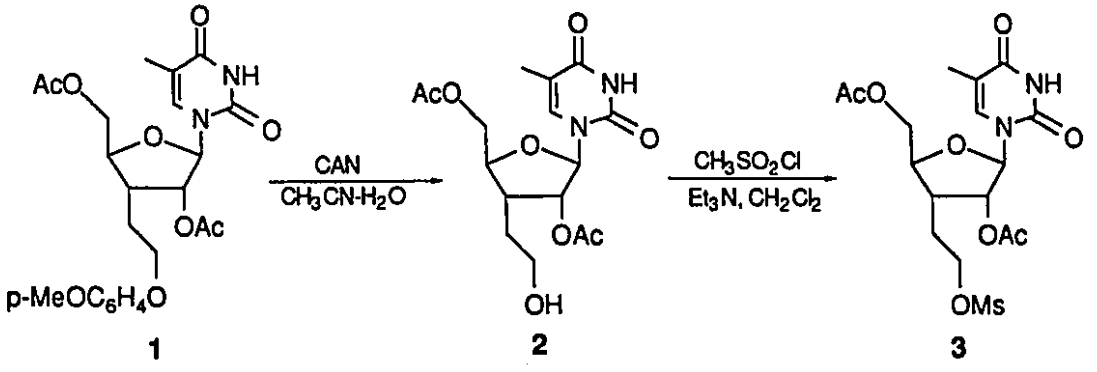
61 Kawai, S. H.; Chin, J.; Just, G.: *Carbohydr. Res.*, **211**, 245 (1991).

62 I thank Dr. Tibor Brenier, Biochem Pharma Inc, for making a large amount of **S6** available to me.

triethylamine, produced mesylate **3** in 97%. The assignment of ^{1}H-NMR spectrum (Figure 13) for compound **3** was done by comparing it with that for alcohol **2**. The singlet at 3.02 ppm was assigned to the methyl function of the methanesulfonyl substitutent. The structure of mesylate **3** was also confirmed by ^{13}C-NMR and mass spectrometry.

Scheme 6



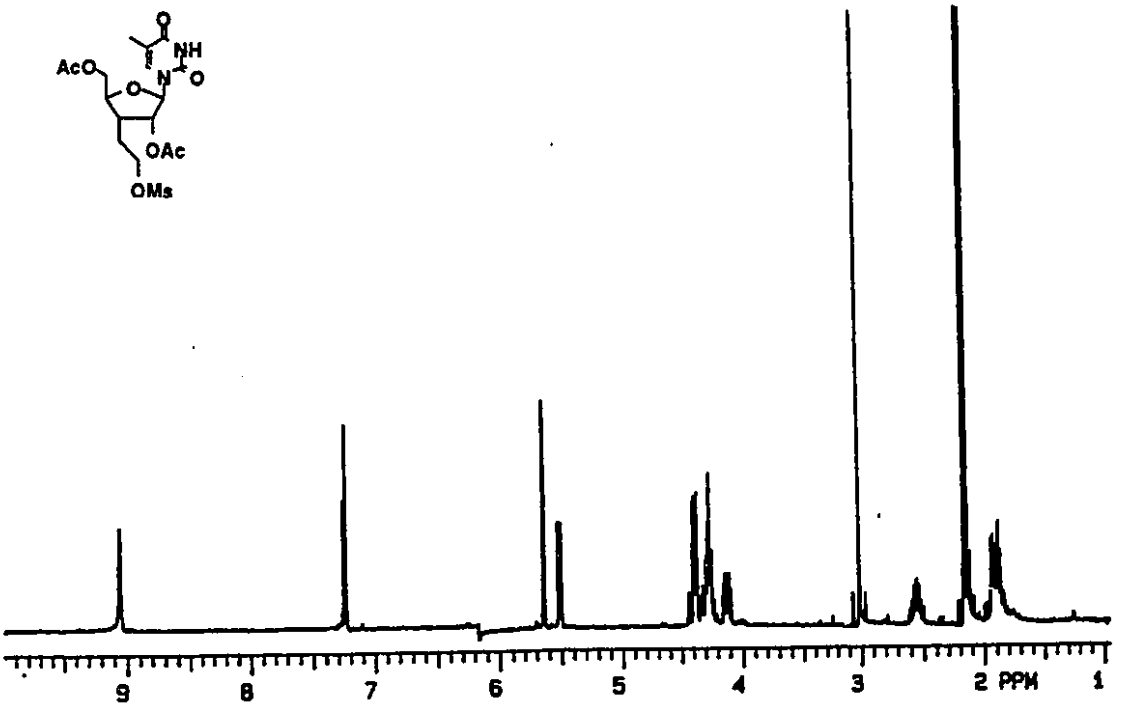


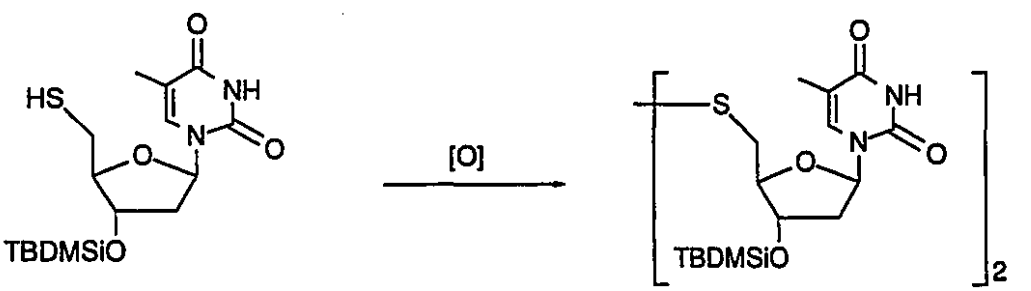
Figure 13 The 200 MHz ^{1}H-NMR spectrum of mesylate **3** in $CDCl_3$.

2.2 Synthesis of Dimer (9)

Coupling reaction of mesylate **3** and thiol **4** was carried out in the presence of cesium carbonate in dry DMF under nitrogen (Scheme 8)[63]. We found that the cleanest result was obtained by saturating the mixtures of cesium carbonate in dry DMF and reactants in dry DMF with nitrogen over a period of 20 minutes, respectively. Otherwise, the formation of a by-product, presumably the symmetrical disulfide resulting from the oxidation of thiol **4** (Scheme 7), reduced the yield of **5**. Chromatography of crude product afforded dimer **5** in 84% yield. Dimer **5** was then deacylated using methanolic ammonia giving diol **6**.

Figure 14 shows the 300 MHz ^{13}C-NMR spectrum of dimer **5** in $CDCl_3$, and figure 15 the 500 MHz ^{1}H-NMR of diol **6** in D_2O. The structures of **5** and **6** were also confirmed by mass spectrometry.

Scheme 7

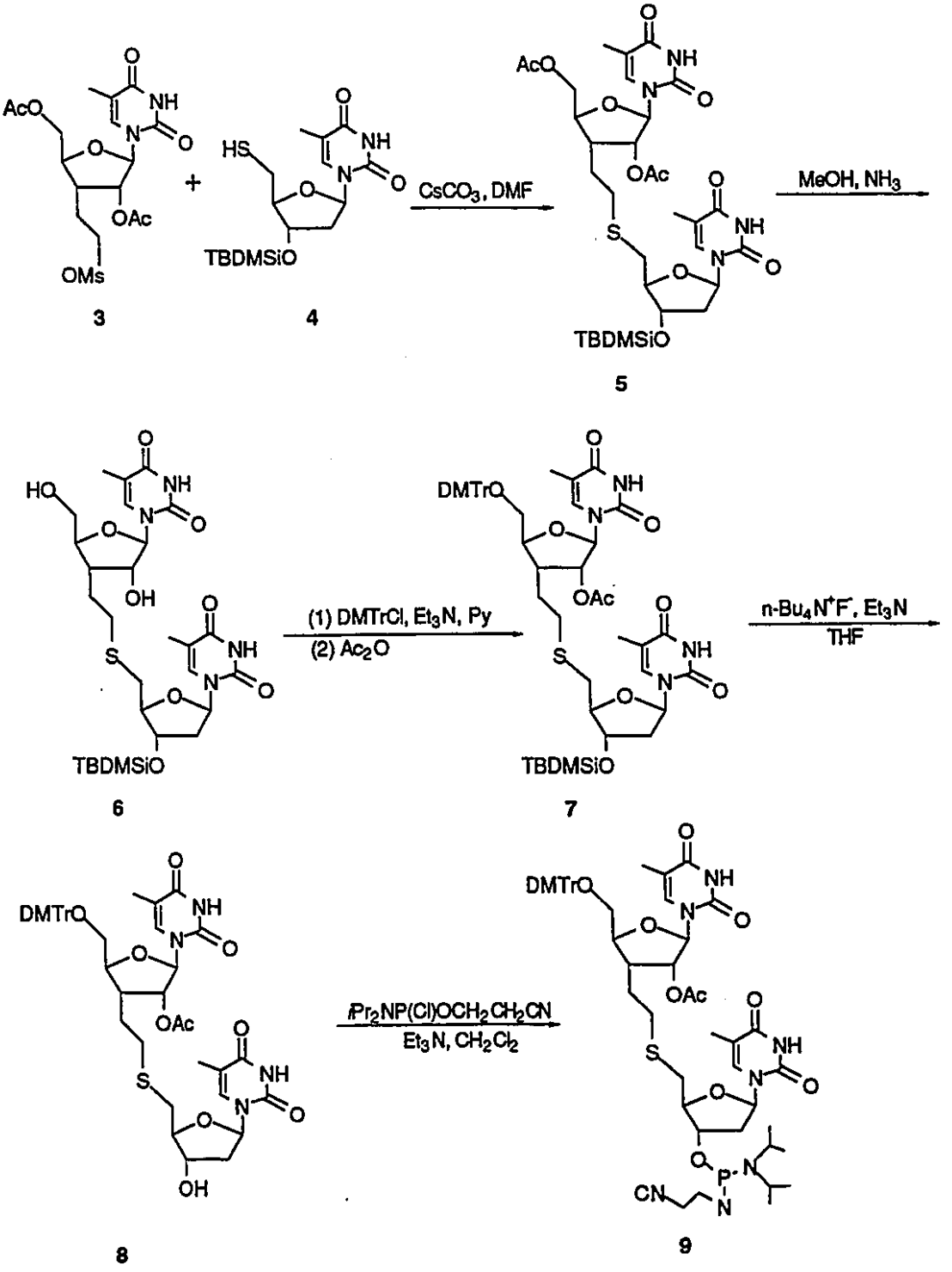


Thiol Disulfide

The selective functionalization of 5'-primary alcohol of **6** and the reacetylation of the 2'-OH group were performed in a one-pot reaction. In order to avoid the formation of 2',5'-ditritylated by-product, the method

63 Vriesema, B. K.; Lemarie, M.; Buter, J.; Kellogg, R. M.: *J. Org. Chem.*, **51**, 5169 (1986).

Scheme 8


AcO
OAc
OMs
3
HS
TBDMSiO
4
$CsCO_3$, DMF
MeOH, NH_3
5
HO
OH
6
(1) DMTrCl, Et_3N, Py
(2) Ac_2O
DMTrO
7
n-$Bu_4N^+F^-$, Et_3N
THF
8
$iPr_2NP(Cl)OCH_2CH_2CN$
Et_3N, CH_2Cl_2
9

described by Hakimelahi *et al*[64] was adopted. Thus, diol **6** was dissolved in dry pyridine at room temperature, and dimethoxytrityl chloride and triethylamine were added in three equal portions over a period of 8 hour. Detection by tlc indicated that only a trace amount of ditritylated compound was formed. After completion of the dimethoxytritylation, acetic anhydride was added to protect the 2'-hydroxyl group. This acetate group was stable enough to successive desilylation of the 3'-silyl function, yet labile enough to be removed by NH_3 in the final deprotection procedure. The crude product was applied to a short column with a mixture of MeOH, CH_2Cl_2 and Et_3N as the eluent. The use of Et_3N was essential to avoid cleavage of the dimethoxytrityl group. The resulting dimethoxytrityl ether **7** was obtained in 76% yield.

The 3'-silyl ether was then cleaved with tetra-*n*-butylammonium fluoride (TBAF) in THF containing Et_3N in 94% yield, and the resulting alcohol **8** was treated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite under standard condition[65] to yield the appropriately activated hydroxy sulfide dimer **9** as a mixture of diastereomers at phosphorus. This product was used after purification on silica gel (CH_2Cl_2/MeOH/Et3N, 100:5:1 v/v) for incorporation into a DNA strand.

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

65 Gait, M. J.: *Oligonucleotide Synthesis: A Practical Approach*, IRL Press, Oxford (1984).

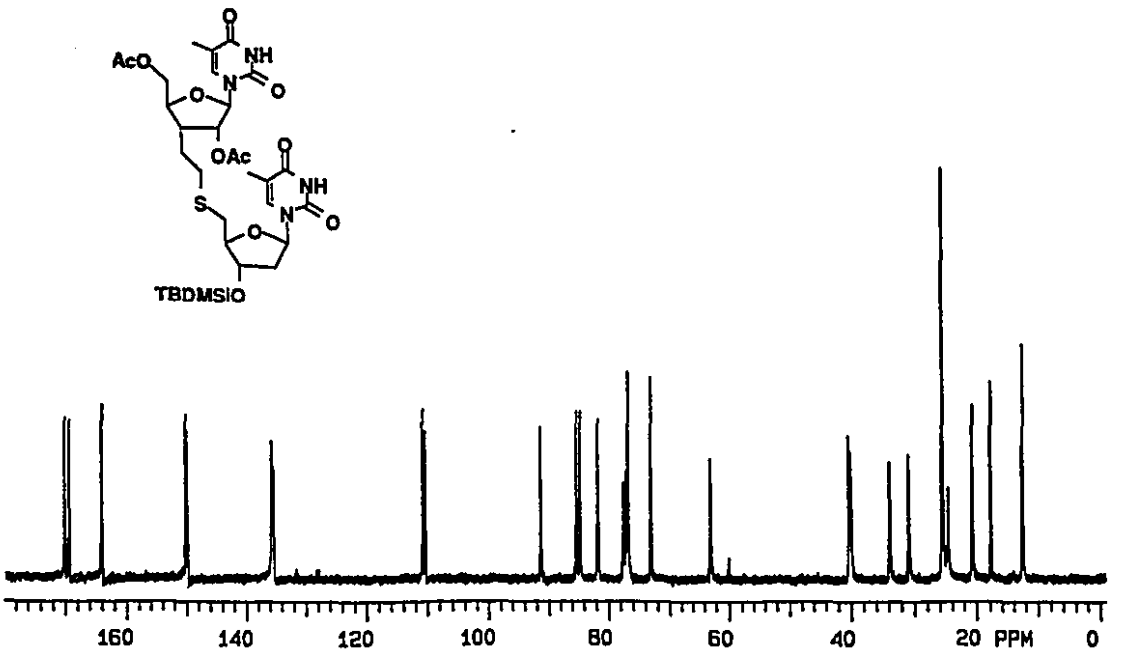


Figure 14 The 300 MHz ^{13}C-NMR spectrum of dimer **5** in $CDCl_3$.

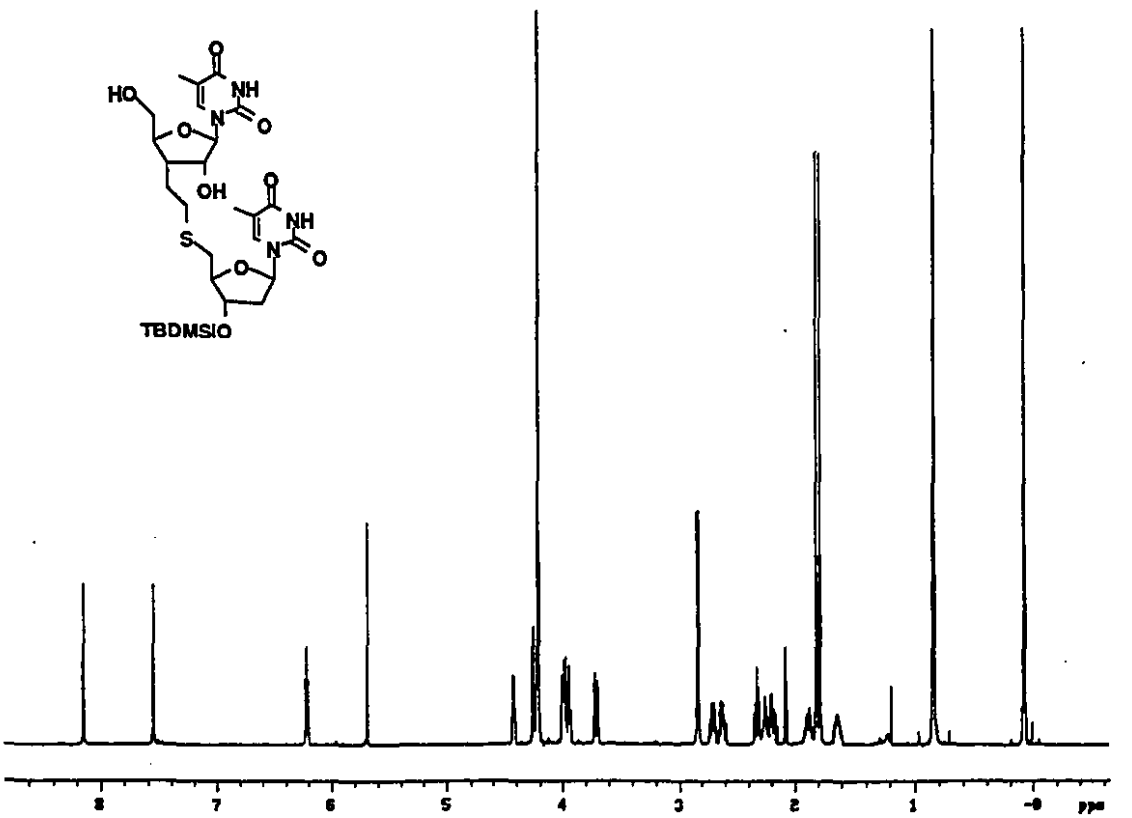
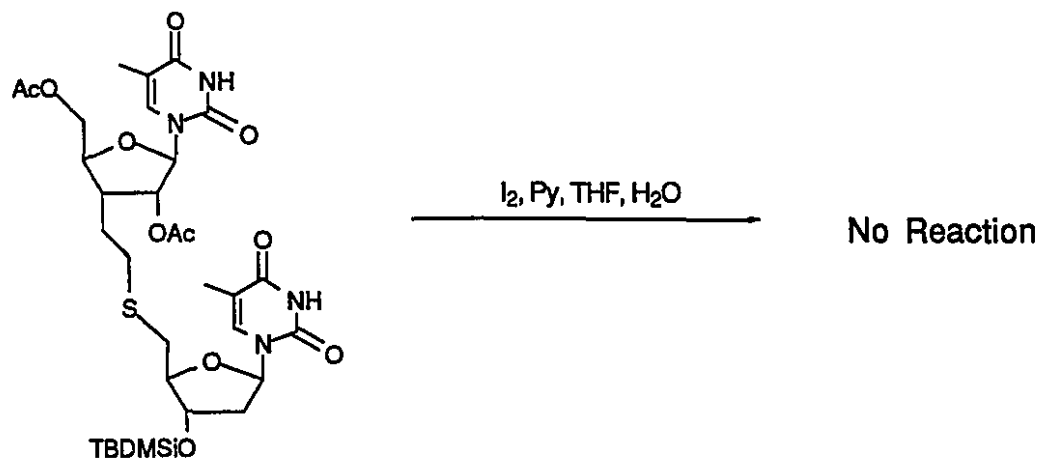


Figure 15 The 500 MHz ^{1}H-NMR spectrum of diol **6** in D_2O.

2.3 The Stability of Sulfide Bridge towards Iodine

To verify that the sulfides incorporated into the DNA strand are not converted to the sulfoxide or sulfone during the oxidation step of the cycle, a sample of sulfide dimer **5** was stirred in the reagent I_2/pyridine/THF/H_2O for 15 minutes (30 times longer than that in the oligomer synthetic cycle). Reference sulfoxides and sulfone were obtained by oxidation of **5** with buffered oxone reagent ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$). Thin-layer chromatography of **5** treated with I_2 and of the sulfoxides and sulfone dimers obtained, clearly demonstrated that no oxidation had taken place (Scheme 9).

Scheme 9

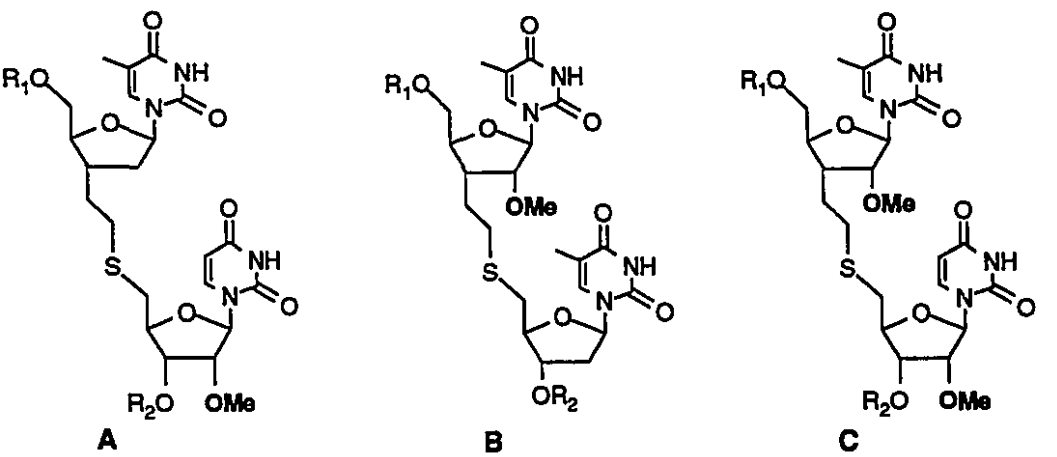


5

Chapter 3. Synthesis of Monomethoxy Sulfide Dimer (21)

Three dimers containing an alkyl sulfide linkage with 2'-O-methyl groups were prepared with the hope to find out the best one. Dimer **A** (Scheme 10), in which methoxy group is positioned at the 5'-end, has been synthesized by Wang in our group. Dimer **B** contains the methoxy group at the 5'-end, and dimer **C** contains two methoxy groups at both the 3'- and 5'-ends.

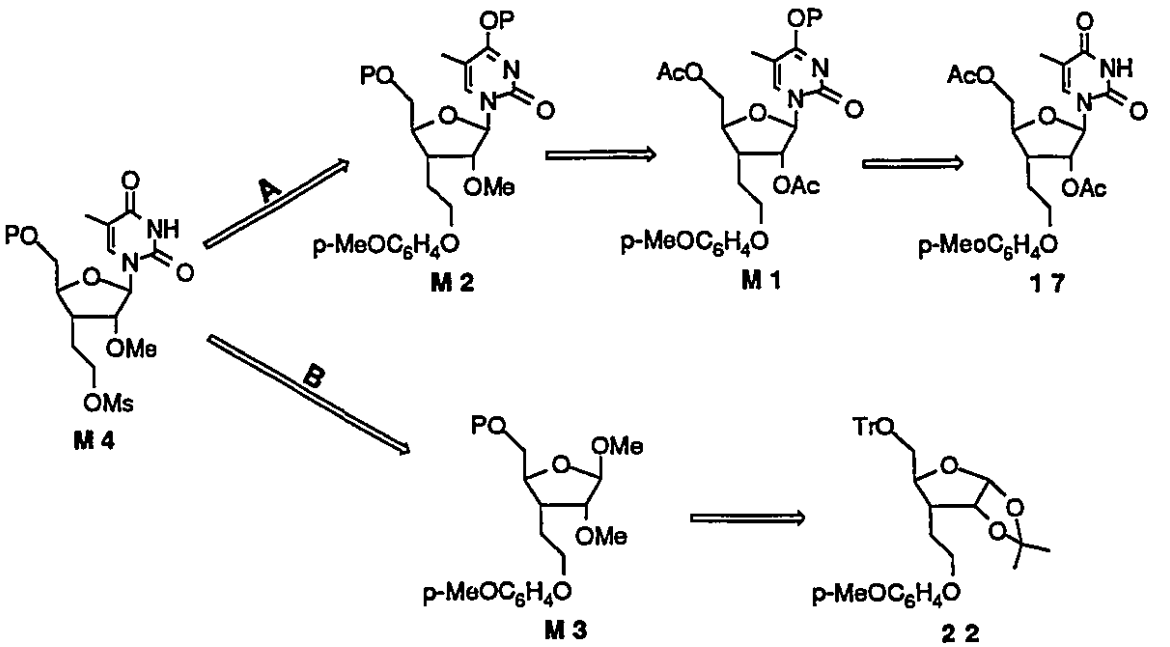
Scheme 10



A **B** **C**

In principle, dimers **B** and **C** can be prepared from the same mesylate **M4** and a different thiol. Two possible routes to get the key intermediate **M4** were investigated and are shown in Scheme 11. In route A, the 3-NH group has to be prevented from reacting with the alkylating agent required to transform the 2'-hydroxyl group to the 2'-methoxy group. For the purpose of obtaining all purine and pyrimidine nucleosides, another way to reach **M4**, which started from sugar derivative **22** as shown in route **B**, was also investigated.

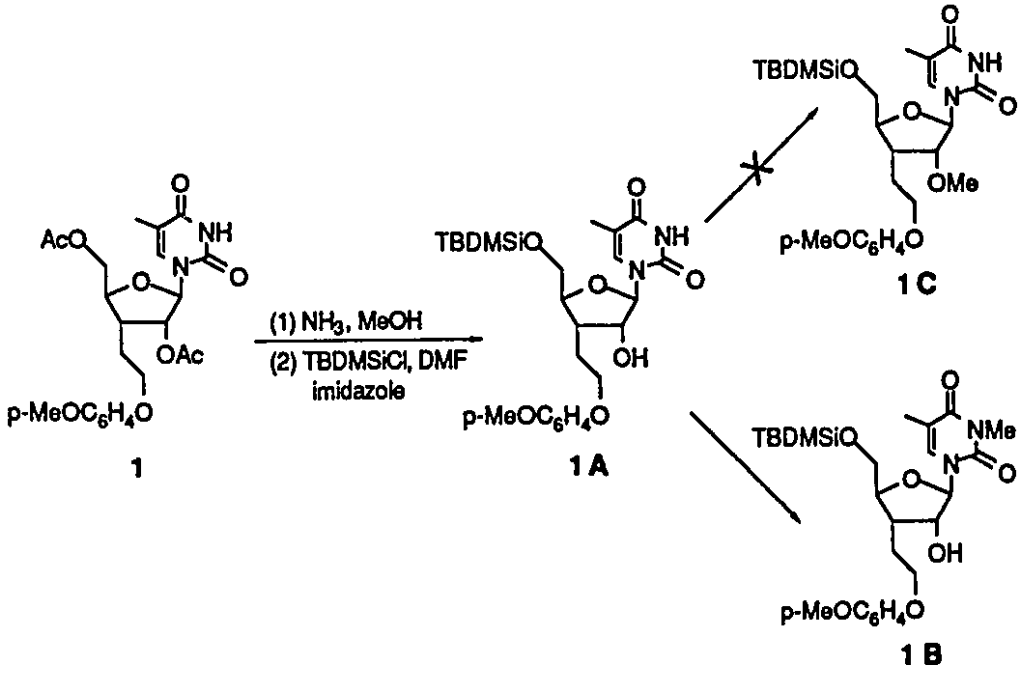
Scheme 11


PO
NH
N
O
OMe
OMs
M 4
A
B
OP
PO
OMe
p-MeOC6H4O
M 2
AcO
OAc
M 1
17
TrO
M 3
22

3.1 Protection of Nucleoside (1)

Compound **1A** was initially used since it was available from other work being carried out in the laboratory. Attempting methylation of the 2'-hydroxyl group of compound **1A** with sodium hydride and methyl iodide in CH_2Cl_2 was unsuccessful. The major product was determined to be as N-methylthymidine **1B** (Scheme 12), rather than the expected 2'-O-methylthymidine **1C**. ^{1}H-NMR offered evidence to confirm its structure because of the absence of the N-H peak of **1C**. Thus, the nucleoside base of **1** must be protected in order to have a better control in the methylation reaction.

Scheme 12



Several protecting groups for nucleoside bases have been recommended in some oligonucleotide syntheses. They include O-phenyl[66],

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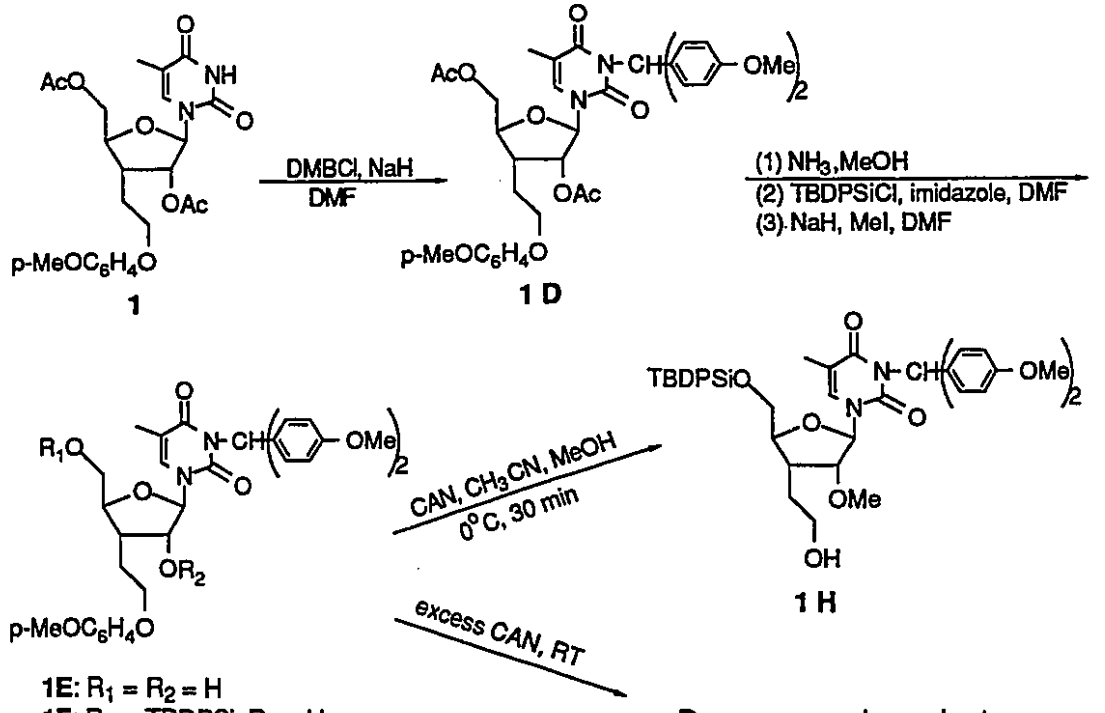
66 Reese, C. B.; Skone, P. A.: *J. Chem. Soc. Perkin Trans.*, I, 1263 (1984).

Scheme 13



2,2,2-trichloro-*t*-butyloxycarbonyl[67], *p*-nitrophenylethyl[68], and methyloxyethoxymethyl (MEM)[69]. 4,4'-Dimethoxyphenylmethyl chloride[70] (dimethoxybenzhydryl chloride), which was prepared by stirring 4,4'-dimethoxyphenylmethanol in diethyl ether with concentrated HCl overnight[71], was initially explored as N-protecting group for nucleoside **1** (Scheme 13). This protecting group was chosen since it may be oxidative

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67 Kamimura, T.; Masegi, T.; Hata, T.: *Chem. Lett.*, 965 (1982).

68 Pfleiderer, W.; Himmelsbach, F.; Charubala, R.; Schirmeister, H.; Beiter, A.; Schulz, B.; Trichtinger, T.: *Nucleosides Nucleotides*, **4**, 81 (1985).

69 Ito, T.; Ueda, S.; Takaku, H.: *J. Org. Chem.*, **51**, 931 (1986).

70 AbuSbeih, K.; Bleasdale. C.; Golding, B. T.; Kitson, S. L.: *Tetrahedron Lett.*, **33**, 4807 (1992).

71 Hanson, R. W.; Law, H. D.: *J. Chem. Soc.*, 7285 (1965).

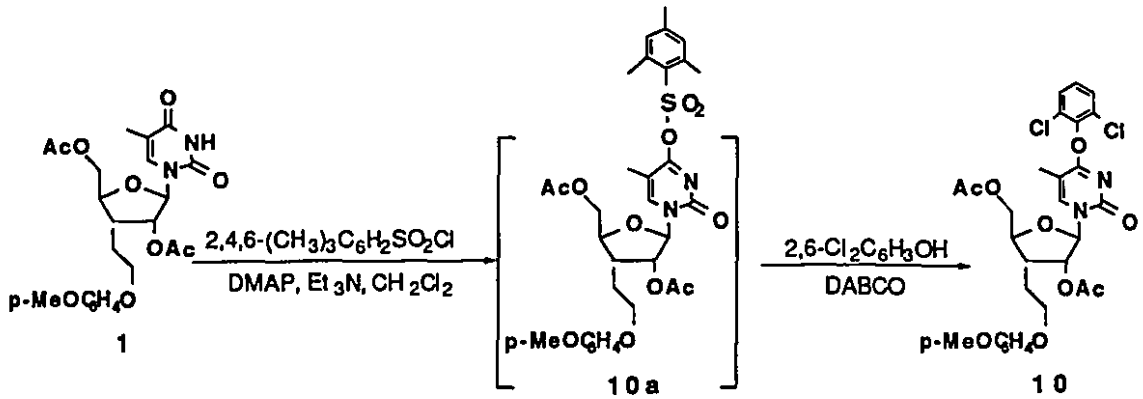
removed under the same conditions as the *p*-methoxyphenyl group at the 2"-position of the nucleoside **1**.

A solution of **1** and dimethoxyphenylmethyl chloride in THF containing sodium hydride was refluxed for 62 hour to afford **1D**. The structure of **1D** was confirmed by ^{13}C-NMR (Figure 16). Stirring compound **1D** in methanolic ammonia gave the diol **1E**, which upon silylation afforded **1F**. The reason to use the *t*-butyldiphenylsilyl and not the *t*-butyldimethylsilyl group will be discussed in section 3.2. Then alcohol **1F** was converted to its corresponding methyl ether **1G** in the usual manner (NaH/MeI/DMF). Unfortunately, the oxidative removal of the N/O-protecting groups with ceric ammonium nitrate (CAN) in acetonitrile-methanol did not proceed to completion in a short time (30 minutes) at 0°C. Only the O-*p*-methoxyphenyl group (compound **1H**) was cleaved under these conditions. The use of excess CAN resulted in the formation of many unidentified products.

However, a 2,6-dichlorophenyl group[72] as a protection group was easily introduced and removed under mild conditions. The 2,6-dichlorophenyl derivative was prepared in the following manner (Scheme 14). Nucleoside **1** was reacted with 2-mesitylenesulfonyl chloride in CH_2Cl_2 containing triethylamine and *N,N*-dimethylaminopyridine to provide the intermediate **10a**, which was not isolated. To the above solution 2,6-dichlorophenol and 1,4-diazabicyclo[2,2,2]octane were added, resulting in the formation of the displacement product, O^4-(2,6-dichlorophenyl)-diacetate **10**, in 85% yield. The ^{13}C-NMR spectra of compounds **1**, **1D** and **10** are shown in Figure 16 and demonstrate the difference in the substitution pattern. The C-5 carbon of **10** is found at 103.50 ppm,

72 Sproat, B. S.; Beijer, B.; Iribarren, A.: *Nucl Acids Res.*, **18**, 41 (1990).

Scheme 14



those of compound **1** and **1D** are at 110.63 and 110.03 ppm, respectively. These assignments agree with those reported for O^4 and N^3-acylated uridine derivatives[73], which indicated that the C5 signal appears at around 101 ppm for unsubstituted and N^3-substituted uridine, and at around 96 ppm for the O^4-substituted uridine.

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73 Kamimura, T.; Masegi, T.; Sekine, M.; Hata, T.: *Tetrahedron Lett.*, **25**, 4241 (1984).

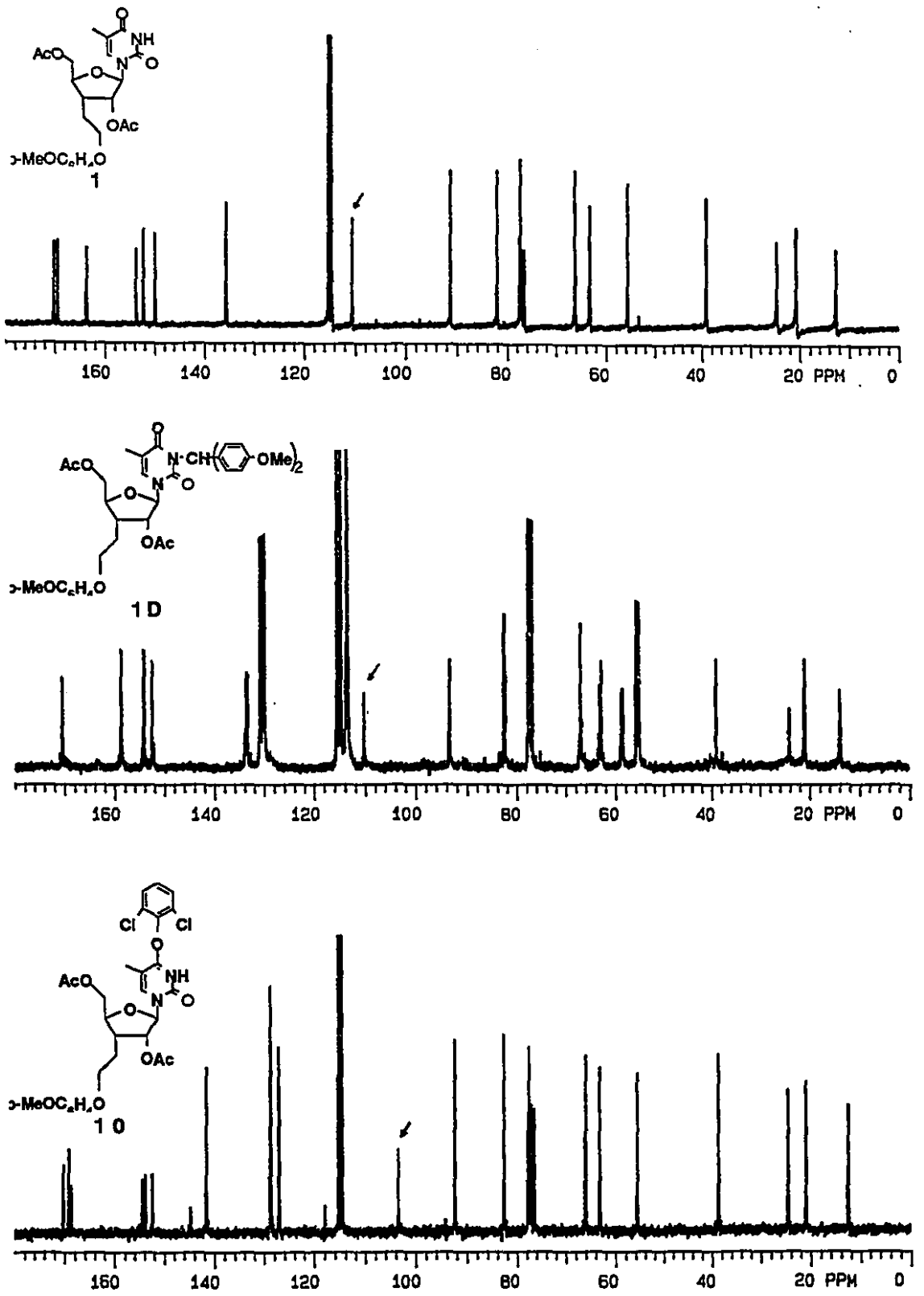
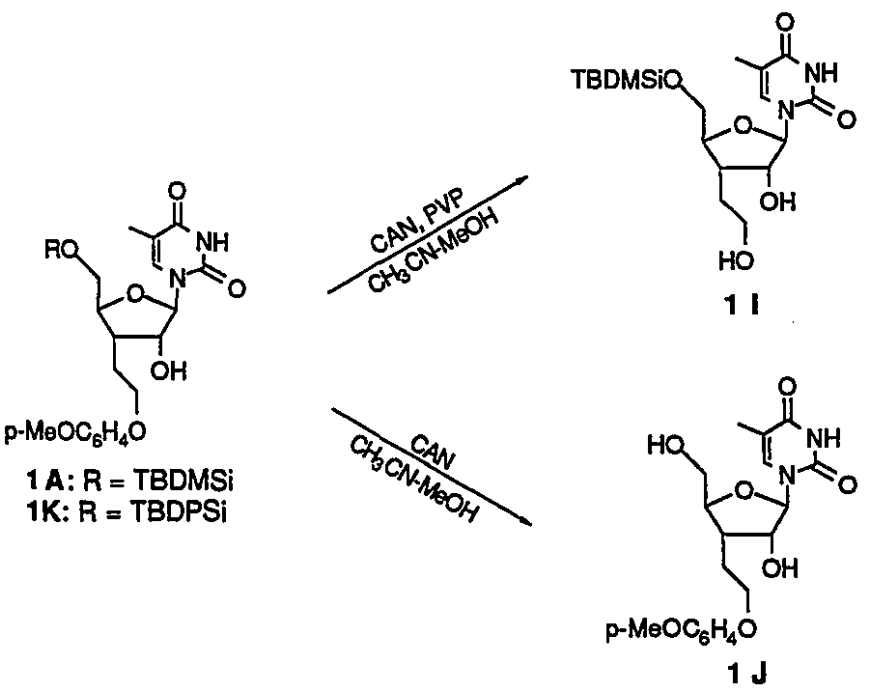


Figure 16 The 300 MHz ^{1}C-NMR spectra of compounds **1**, **1D** and **10**.

3.2 Synthesis of Mesylate (16)

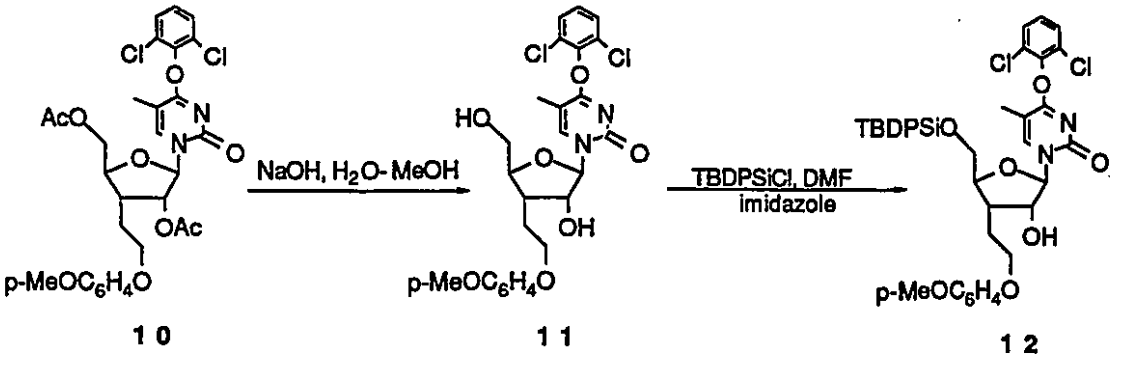
After protection of the base, O^4-(2,6-dichlorophenyl)diacetate **10** was hydrolyzed with sodium hydroxide in H_2O-MeOH (Scheme 16) and afforded O^4-(2,6-dichlorophenyl)diol **11** in 91% yield. The following step was to select a proper protecting group which could selectively react with the 5'-hydroxyl group and be stable under mild acidic condition required by the cleavage of the 2"-*p*-methoxyphenyl group using cerium ammonium nitrate. Trityl, *t*-butyldimethysilyl or *t*-butyldiphenylsilyl groups are quite selective for a primary alcohol. The first one is acid labile, so that attention was paid to the latter two. Since *t*-butyldimethylsilyl chloride is cheaper than *t*-butyldiphenylsilyl chloride, compound **1A** was initially used as a model compound to survey its stability towards ceric ammonium nitrate (CAN) deprotection (Scheme 15). Treatment of **1A** with CAN in

Scheme 15



CH_3CN-H_2O or CH_3CN-MeOH gave predominantly desilylated product **1J**, as detected by tlc. In order to overcome the desilylation, poly-(4-vinylpyridine-co-styrene) (PVP)[74] was introduced in the solution of CH_3CN-MeOH containing **1A** and CAN. The resulting alcohol **11** was obtained in 62% yield in a small scale preparation, but the reaction was hard to handle on a large scale. However, compound **1K**, which was similar to **1A** except for the *t*-butyldiphenylsilyl group at the 5'-position, could be used in the CAN reaction (CAN, CH_3CN-MeOH) and was stable to the reaction conditions. Thus, *t*-butyldiphenylsilyl was chosen to protect O^4-(2,6-dichlorophenyl)-diol **11** (Scheme 16), and the reaction was performed under standard conditions (TBDPSiCl/imidazole/DMF). O^4-(2,6-Dichlorophenyl)-5'-silylether **12** was produced in 81% yield.

Scheme 16



10 **11** **12**

The methylation of the 2'-hydroxyl group of **12** was carried out with sodium hydride and methyl iodide in DMF at 0°C[75] (Scheme 17). We found

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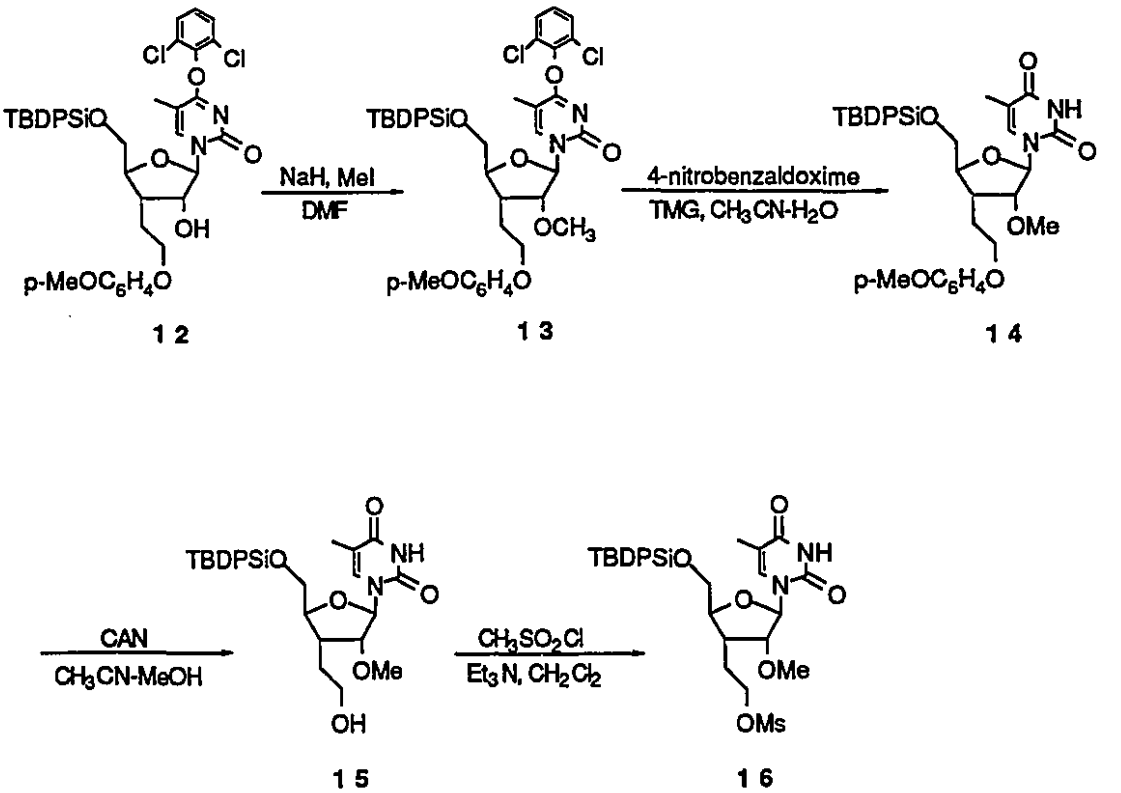
74 McKllop, A; Young, D.: *Syn. Comm.*, **7**, 467 (1977).

75 Quaedflieg, P. J. L. M.; van der Heiden, A. P.; Koole, L. H.; Coenen, A. J. J. M.; van der Wal, S. Meijer, E. M.: *J. Org. Chem.*, **56**, 5846 (1991).

Scheme 17



12 13 14 15 16

that successive addition of sodium hydride and methyl iodide gave the best result, O^4-(2,6-dichlorophenyl)-2'-O-methyl ether **13** being obtained in 98% yield.

The 2,6-dichlorophenyl group was then removed by reaction with 10 equivalents of 4-nitrobenzaldoxime and 1,1,3,3-tetramethylguanidine (TMG) in acetonitrile-methanol[76]. The large excess of 4-nitrobenzaldoxime and TMG was easily removed by washing the reaction solution with water. Chromatography of the crude product gave 2'-O-methyl ether **14** (85%). The structure of compound **14** was confirmed by ^{13}C- and ^{1}H-NMR. The chemical shift of the C-5 carbon of **14** appeared at

76 Reese, C. B.; Titmas, R. C.; Yau, L.: *Tetrahedron Lett.*, **30**, 2727 (1978).

110.15 ppm, which was similar to that of **1** (110.63 ppm), providing proof of successful deprotection. The chemical shift of the 2'-O-methyl protons at 3.58 ppm was in agreement with a similar compound described[77].

The oxidative cleavage of the *p*-methoxyphenyl group at 2"-end of **14** using CAN in acetonitrile-methanol produced alcohol **15** in 72% yield. Mesylation of the alcohol **15** with methanesulfonyl chloride and Et_3N in CH_2Cl_2 afforded mesylate **16** in 87% yield.

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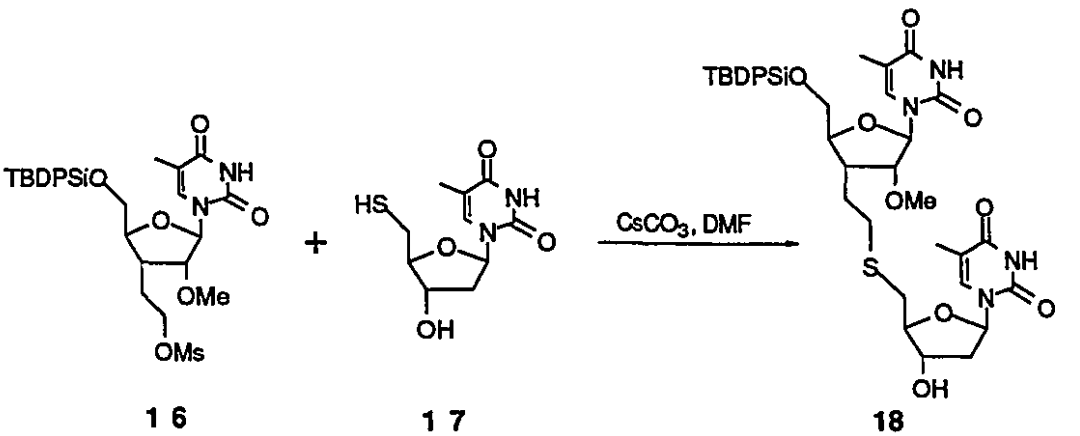
77 Wang, D. G.: *Ph. D. Thesis*, McGill University, (1993).

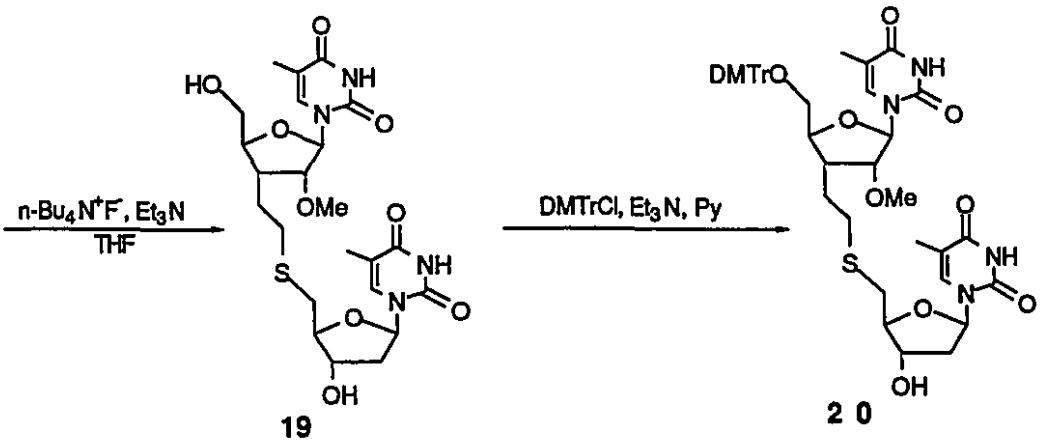
3.3 Synthesis of Monomethoxy Sulfide (21)

The synthetic procedures to obtain phosphoramidite **21** started from mesylate **16** and thiol **17** and were similar to the syntheses of hydroxy sulfides described in chapter 2. Scheme 18 outlines the procedures. Thiol **17** was used without 3'-protection since the 3'-hydroxyl group did not react during the subsequent functionalization with dimethoxytrityl chloride (DMTrCl). The coupling of mesylate **16** and thiol **17** was carried out in DMF employing cesium carbonate. The solutions of cesium carbonate in DMF and reactants in DMF were deoxygenated by nitrogen over a period of 20 min; this was necessary because, otherwise, rapid oxidation of the thiol to the disulfide under basic conditions did occur. After work up and purification by chromatography, dimer **18** was obtained in 84% yield. The desilylation of **18** by tetra-*n*-butylammonium fluoride in THF gave diol **19** in 81% yield. Figure 17 shows the 300 MHz ^{13}C-NMR of dimer **18** in $CDCl_3$ and Figure 18 shows the 500 MHz ^{1}H-NMR of diol **19** in D_2O. They are consistent with the structures proposed. The 5'-hydroxyl function was then selectively functionalized by DMTrCl in pyridine containing triethylamine, producing dimethoxytrityl ether **20** in 83% yield. When this dimethoxytrityl ether was treated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite in CH_2Cl_2 containing triethylamine, the 3'-hydroxyl group was transformed to the phosphoramidite **21** in 95% yield. ^{31}P-NMR indicated, as expected, the presence of two diastereomeric phosphoramidites, δ 150.85 and 151.03 with respect to aqueous phosphoric acid. Phosphoramidite **21** was used as such for incorporation into DNA.

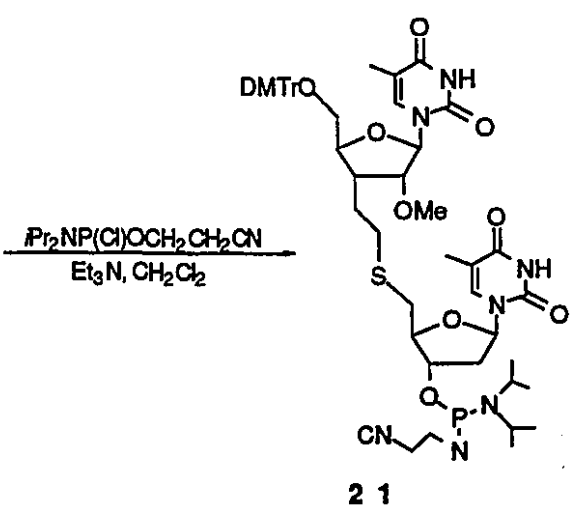
Scheme 18



16 17 18



19 20



21

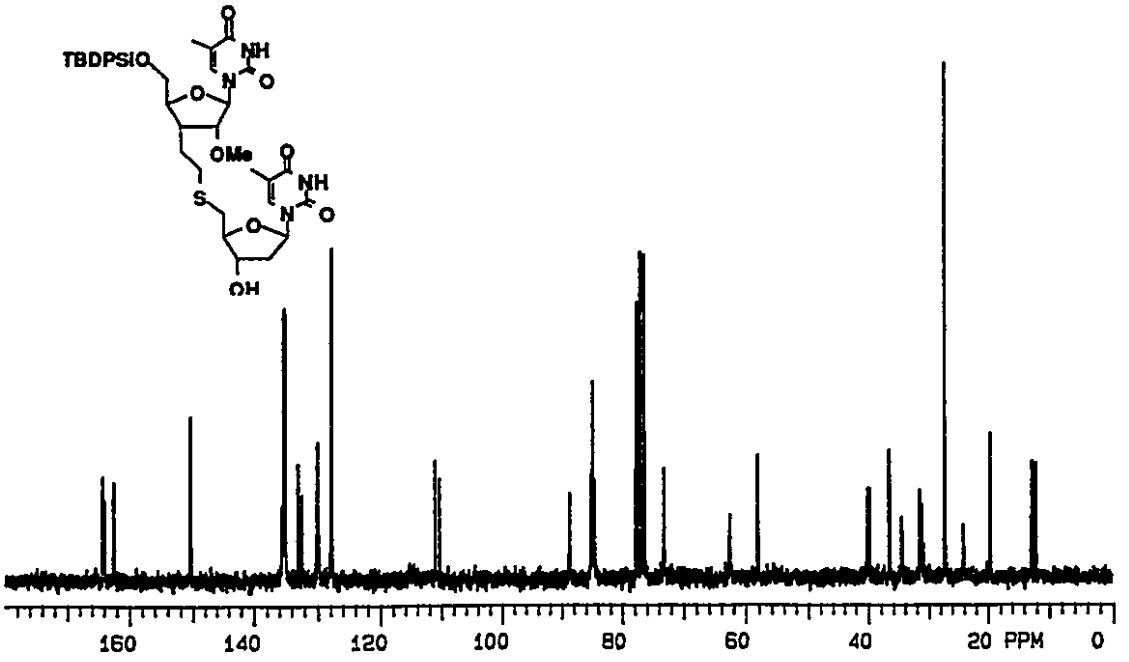


Figure 17 The 300 MHz ^{13}C-NMR spectrum of dimer **18** in $CDCl_3$.

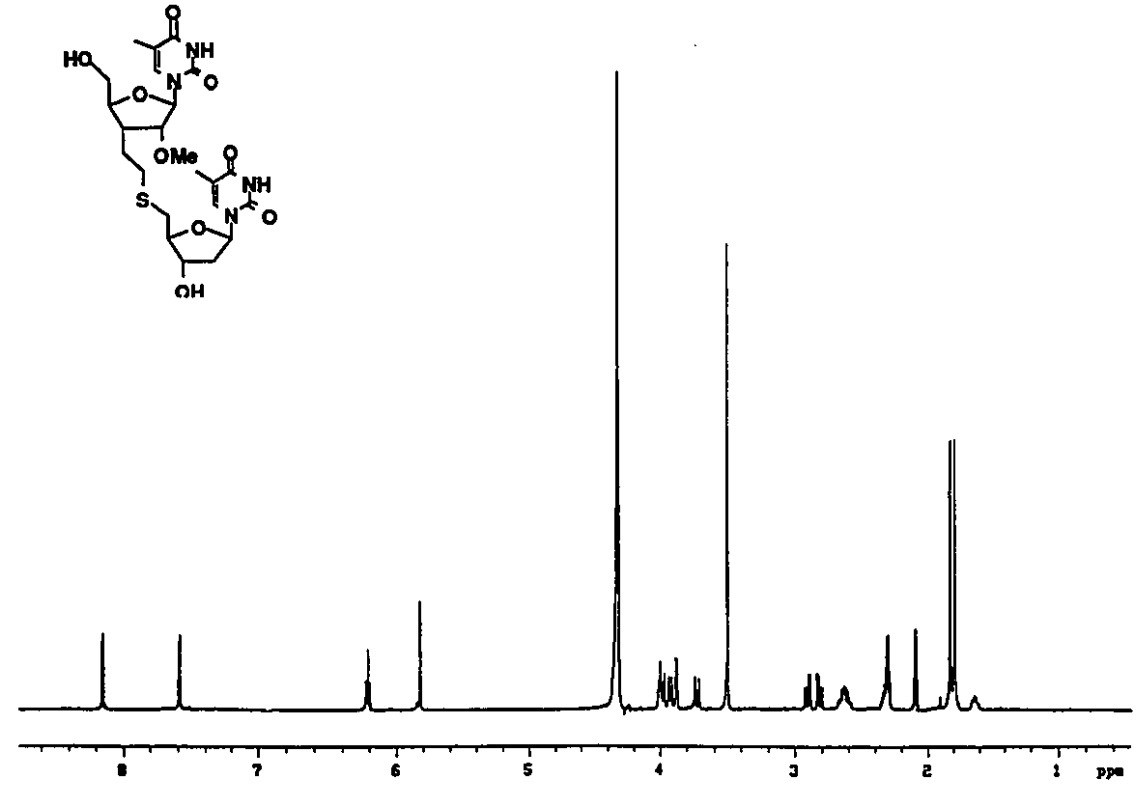


Figure 18 The 500 MHz ^{1}H-NMR spectrum of diol **19** in D_2O.

3.4 The Synthesis of Nucleoside (28) from a Carbohydrate

Another synthetic route to nucleoside **28**, in which the thymine base was introduced after the methylation of the 2-hydroxyl group, was also investigated. 3-Deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-2'-O-*p*-methoxyphenyl-5-trityl-D-ribofuranose **22** was used as a starting material. Its synthesis has been discussed in Chapter 2.

The instability of the trityl group under acidic conditions required its replacement with an acidic stable function. Treatment of trityl ether **22** with trichloroacetic acid in CH_2Cl_2 at room temperature provided alcohol **23** in 71% yield (Scheme 19). The 5'-O-acetate and 5'-O-*t*-butyldimethylsilyl ether of **23**, which could be synthesized in excellent yields, failed to resist the acidic conditions used in the next step. However, the benzyl group was stable to the acidic requirements. It was prepared by the reaction of **23** with sodium hydride and benzyl bromide in THF in the presence of *t*-butylammonium iodide as catalyst[78] . After destroying the remaining sodium hydride by carefully adding water to the reaction solution, the crude product obtained after extraction could be directly used in the next step.

Refluxing benzyl ether **24** in an acidic methanol solution yielded alcohol **25** as a yellow syrup. The trans relationship of H1 and H2 was confirmed by ^{1}H-NMR, which showed a singlet at 4.90 ppm.

Then, the resulting 2-hydroxyl group was methylated employing sodium hydride and methyl iodide, producing 1,2-di-O-methyl sugar derivative **26**. The overall yield for compound **26** from **23**, via **24** and **25**, was 66%.

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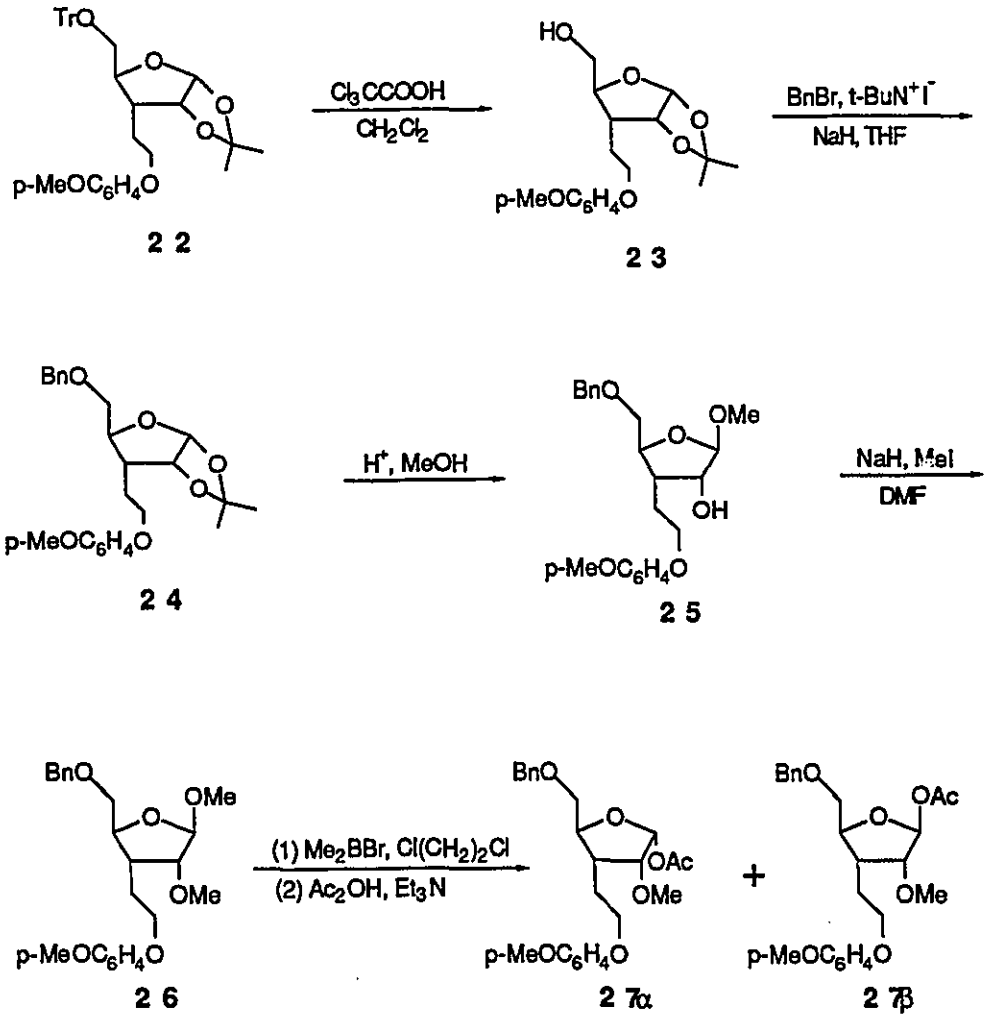
78 Evans, M. E.: *Carbohydr. Res.*, **3**, 453 (1967).

Scheme 19



The subsequent step required the attachment of the base to the sugar. The attempt to convert compound **26** into the thymine nucleoside by activation with dimethylboron bromide, followed by treatment with silylated thymine and trimethylsilytriflate[79], was unsuccessful. Acetal **26** was therefore first transformed to the corresponding 1-O-acetate, the

79 Lavallée, J-F.; Just, G.: *Tetrahedron Lett.*, **32**, 3469 (1991).

starting material for a conventional formation of nucleosides[80]. Acetolysis of **26** with $AcOH/Ac_2O/H^+$ at either high or room temperature did not provide the desired acetate. Thus, this acetate was formed by cleavage of cyclic methyl acetyl of **26** using dimethylboron bromide in 1,2-dichloroethane[81,82], followed by the treatment of acetic acid and triethylamine. Acetate **27** was obtained as a α,β-isomeric mixture in 88% yield.

The structures of α– and β-isomers were determined by ^{1}H-NMR (Figure 19). The major product was assigned as the α-isomer because its H-1 proton appeared a doublet at 6.38 ppm (J = 3.2 Hz), indicating a dihedral angle close to 0°, whereas the singlet at 6.22 ppm was assigned to the β-anomer, which showed a dihedral angle of about 90°. The α to β ratio, as measured by ^{1}H-NMR, was 6 : 1, and the isomers could be separated by column chromatography.

In the formations of nucleosides, the use of catalysts like trimethylsilyl triflate (TMSOTf)[83], $TiCl_4$[84] and $SnCl_4$[85] has become widely accepted. In our case, the reaction of anomeric mixture **27** and silylated thymine in the presence of trimethylsilyl triflate, resulted in the formation of nucleoside **28**αβ in a 1.5 : 1 ratio of α : β. We next tried $TiCl_4$ as catalyst. However, only starting material and decomposition products were obtained. Treatment of **27**α with silylated thymine and redistilled $SnCl_4$ produced nucleoside **28** exclusively as the β-anomer in 48% yield

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80 Vorbrüggen, H.; Krolikiewicz, K.: *Angew. Chem. Internal. Edit.*, **14**, 421(1975).

81 Guindon, Y.; Morton, H. E.; Yoakim, C.: *Tetrahedron Lett.*, **24**, 3969 (1983).

82 Guindon, Y.; Yoakim, C.; Morton, H. E.: *J. Org. Chem.*, **49**, 3912 (1984).

83 Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B.: *Chem. Ber.*, **114**, 1234 (1981).

84 Choi, W.; Wilson, L. J.; Yeola, S.; Liotta, D. C.: *J. Am. Chem. Soc.*, **113**, 9377 (1991).

85 Lichtenthaler, F. W.; Voss, P.; Heerd, A.: *Tetrahedron Lett.*, **24**, 2141 (1974).

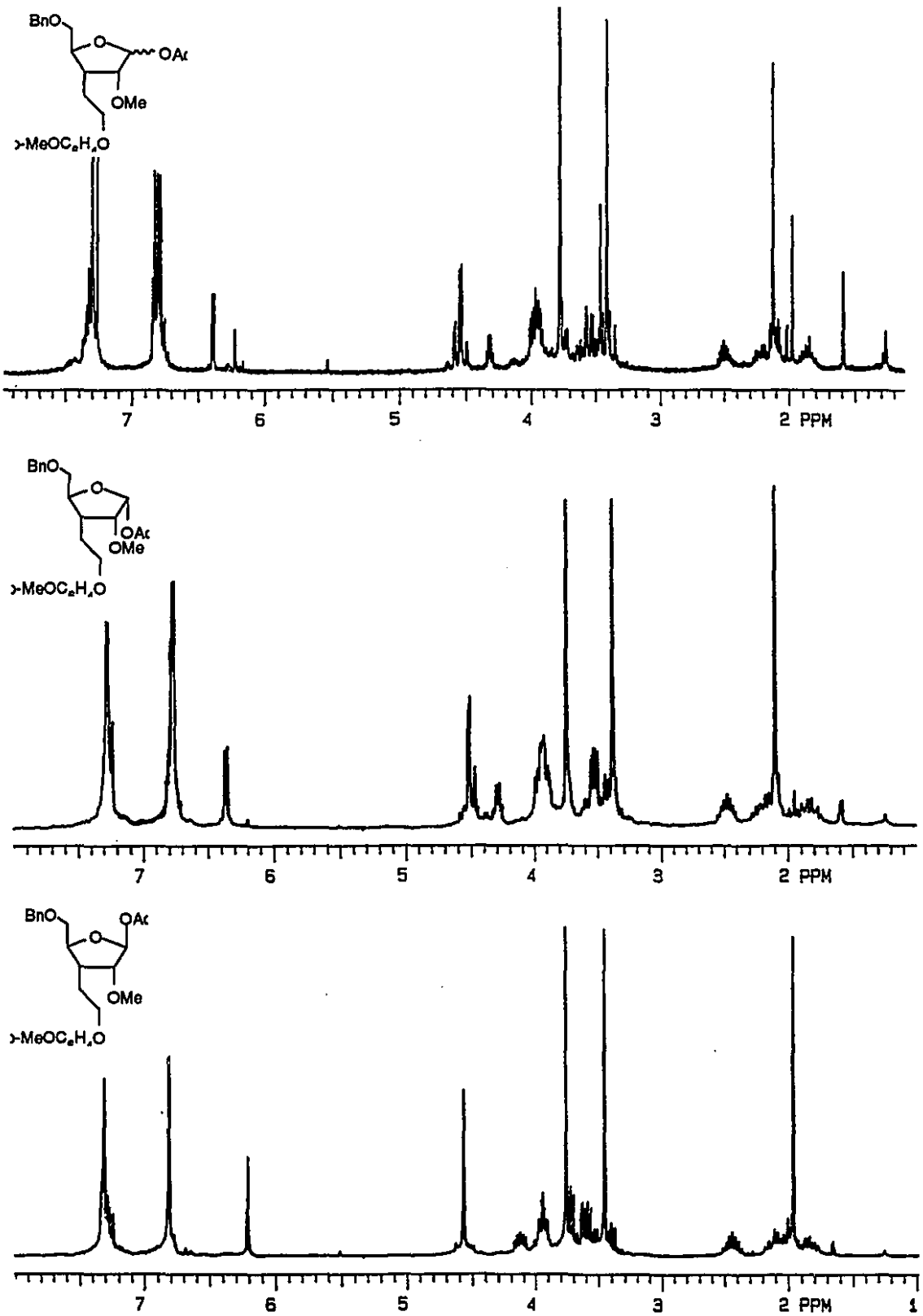
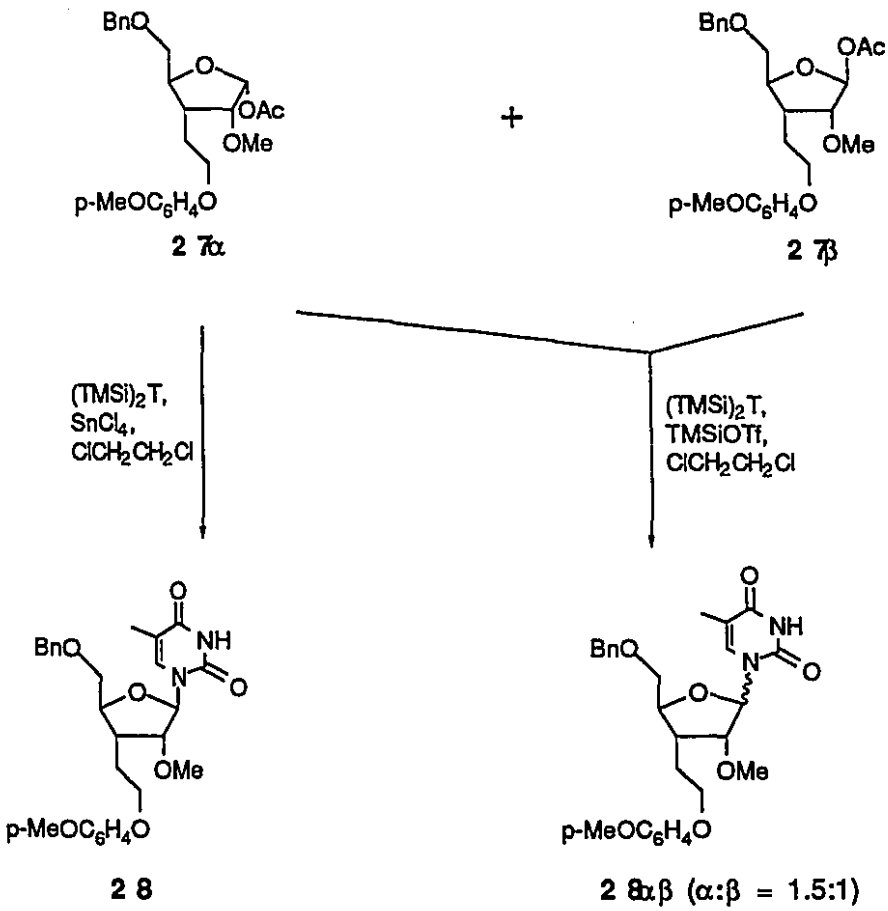
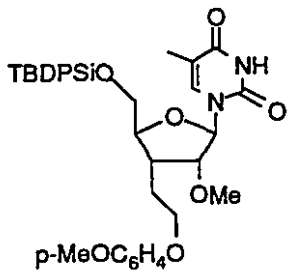


Figure 19 The 200 MHz ^{1}H-NMR spectra of **27**, **27**α and **27**β.

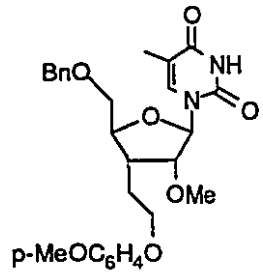
Scheme 20



(Scheme 20). The β-configuration of **28** was confirmed by ^{1}H-NMR, COSY and nOe experiments. ^{1}H and ^{13}C-NMR data for compounds **14** and **28** are very similar and are shown on Table 1 and 2.



1 4



2 8

Tabie 1 ^{1}H-NMR of **14** and **28.**

Compound	5-Me	H1"$_A$	H1"$_B$	H3'	2'-OMe	PhOMe	H1'	H6
1 4	1.47	1.68	2.01	2.60	3.58	3.76	5.93	8.48
2 8	1.51	1.70	2.05	2.52	3.58	3.76	5.91	7.88

Table 2 ^{13}C-NMR **14** and **28.**

Compound	5-Me	C1"	C3'	2'-OMe	C2'	C1'	C5	C6
1 4	11.73	23.59	37.35	57.95	84.93	88.65	110.15	138.31
2 8	11.71	23.60	36.78	57.28	83.26	87.31	109.70	134.41

Chapter 4. Synthesis of Dimethoxy Sulfide (35)

4.1 Modification of the Synthesis of O^2-Methyluridine

The general procedures for the synthesis of O^2-methyluridine were developed by the Nyilas *et al*[86] (Scheme 21). Compound **S9** was easily synthesized in a large quantity by a standard reaction of uridine **S8** with 1,1,3,3-tetraisopropyl-1,3-dichlorodisiloxane in dry pyridine. The key intermediate **S10** was obtained by a one-pot four-step procedure involving 1) trimethylsilylation (TMS) of the 2'-hydroxyl function, 2) conversion to the C-4-(2-mesitylenesulfonate), 3) nucleophilic displacement at the C-4 by the conjugate base of 2-nitrophenol and 4) the removal of the TMS group from the 2'-hydroxyl function by a brief acid treatment. Methylation of the 2'-hydroxyl group produced **S11**, which upon the deprotection of O^4-position (4-nitrobenzaldoxime/TMG/CH_3CN-MeOH) and desilylation of 3'- and 5'-end (TBAF/THF), afforded O^2-methyluridine **29**.

We found the procedure for the synthesis of **S10** could be simplified. We observed that without the protection of the 2'-hydroxyl group by TMS, the attachment of the 2-mesitylenesulfonyl group only occurred at the C-4 position. Thus, the one-pot four-steps procedure could be cut to two-steps. The C-4 substituent of **S10** was confirmed by ^{13}C-NMR, which showed the C-4 peak at 94.32 ppm, compared to uridine around 101 ppm. O^2-Methyluridine **29** obtained in this manner was identical to that reported by Nyilas.

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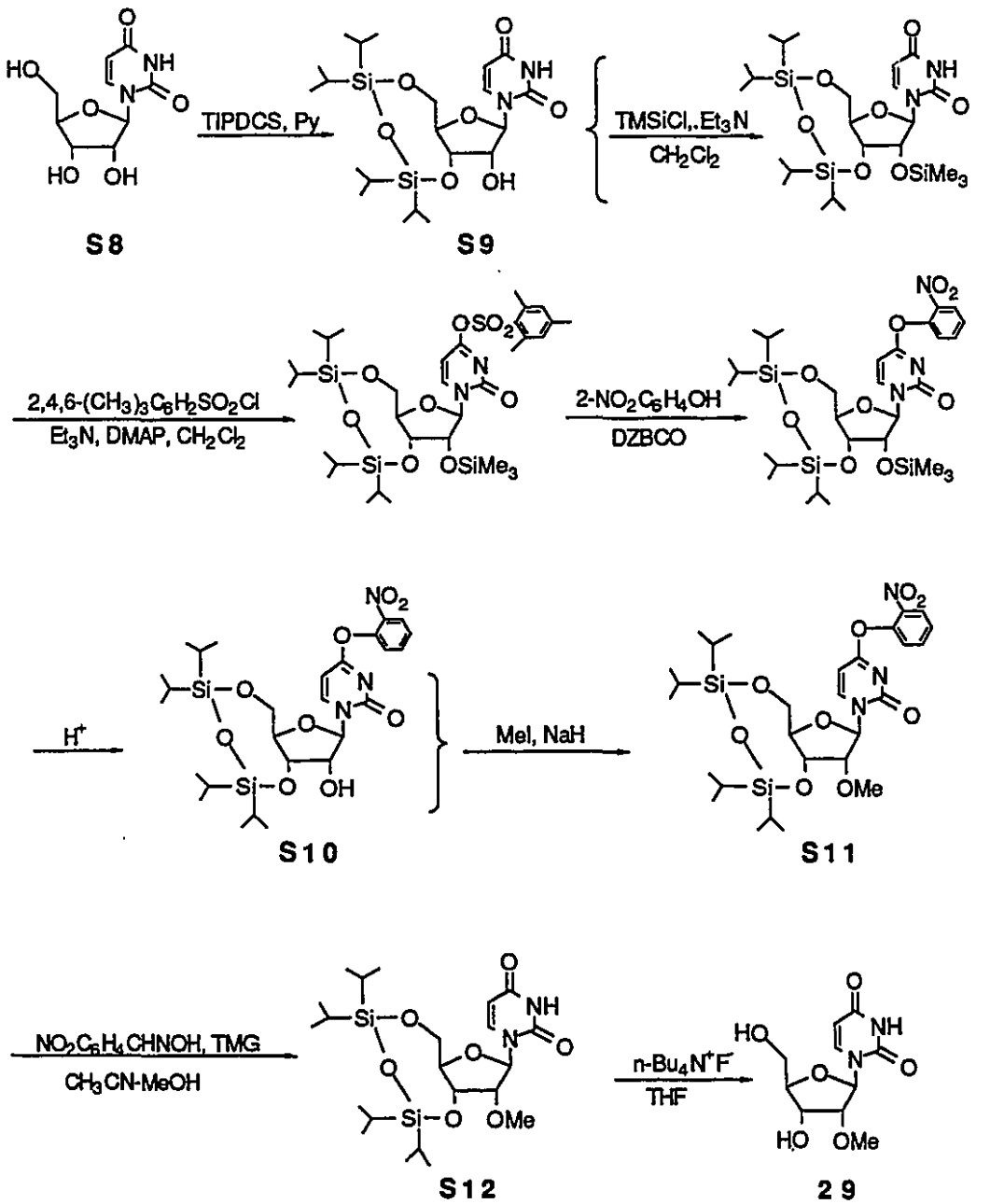
86 Nyilas, A.; Chattopadhyaya, J.: *Acta Chem. Scand.*, **B40**, 826 (1986), and references therein.

Scheme 21

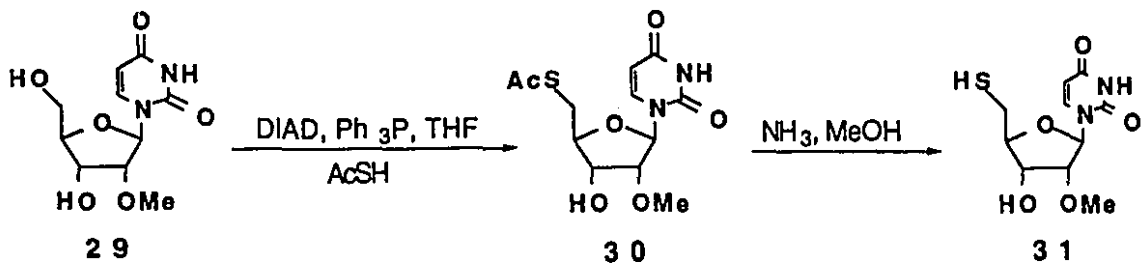


S8 S9 S10 S11 S12 29

4.2 Thiolation of O^2-Methyluridine (29)

O^2-Methyluridine **29** was converted to thioester **30** under Mitsunobu conditions[87,88] (Scheme 22). The Mitsunobu reagents, diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (Ph_3P), were dissolved in THF and initially reacted for 30 minutes at 0°C. To the resulting milky mixture, the solution of **29** and thioacetic acid in THF was slowly added over an one hour period, affording thioester **30** in 84% yield. Treatment of **30** also with deoxygenated methanolic ammonia solution gave thiol **31** in 85% yield.

Scheme 22



29 **30** **31**

87 Mitsunobu, O.: *Synthesis*, 1 (1981).

88 Volante, R. P.: *Tetratedron Lett.*, **22**, 3119 (1981).

4.3 Synthesis of Dimethoxy Sulfide (35)

The condensation of mesylate **16** and thiol nucleoside **31** was performed under the same conditions ($CsCO_3$/DMF) described in chapter 2 (Scheme 23), and proceeded in 80% yield. The 5'-silyl group of the resulting dimer **32** was cleaved using tetrabutylammonium fluoride in THF, and afforded diol **33** in 81% yield. Then the 5'-hydroxyl was treated with dimethoxytrityl chloride and triethylamine, producing dimethoxytrityl ether **34** in 83% yield. Upon the reaction of **34** with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite in dichloromethane, target amidite **35** was obtained after purification by chromatography (CH_2Cl_2 / MeOH / Et_3N, 100:5:1, v/v) in 86% yield.

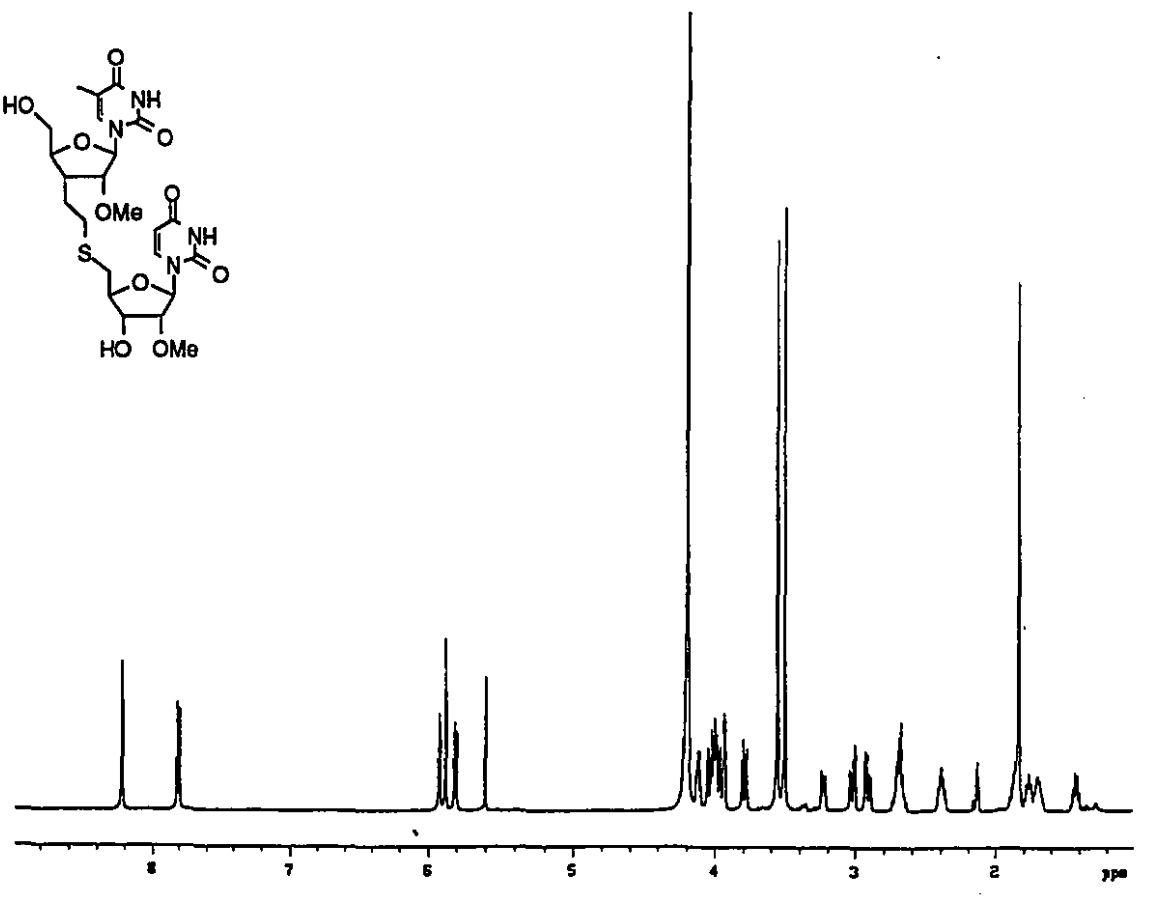
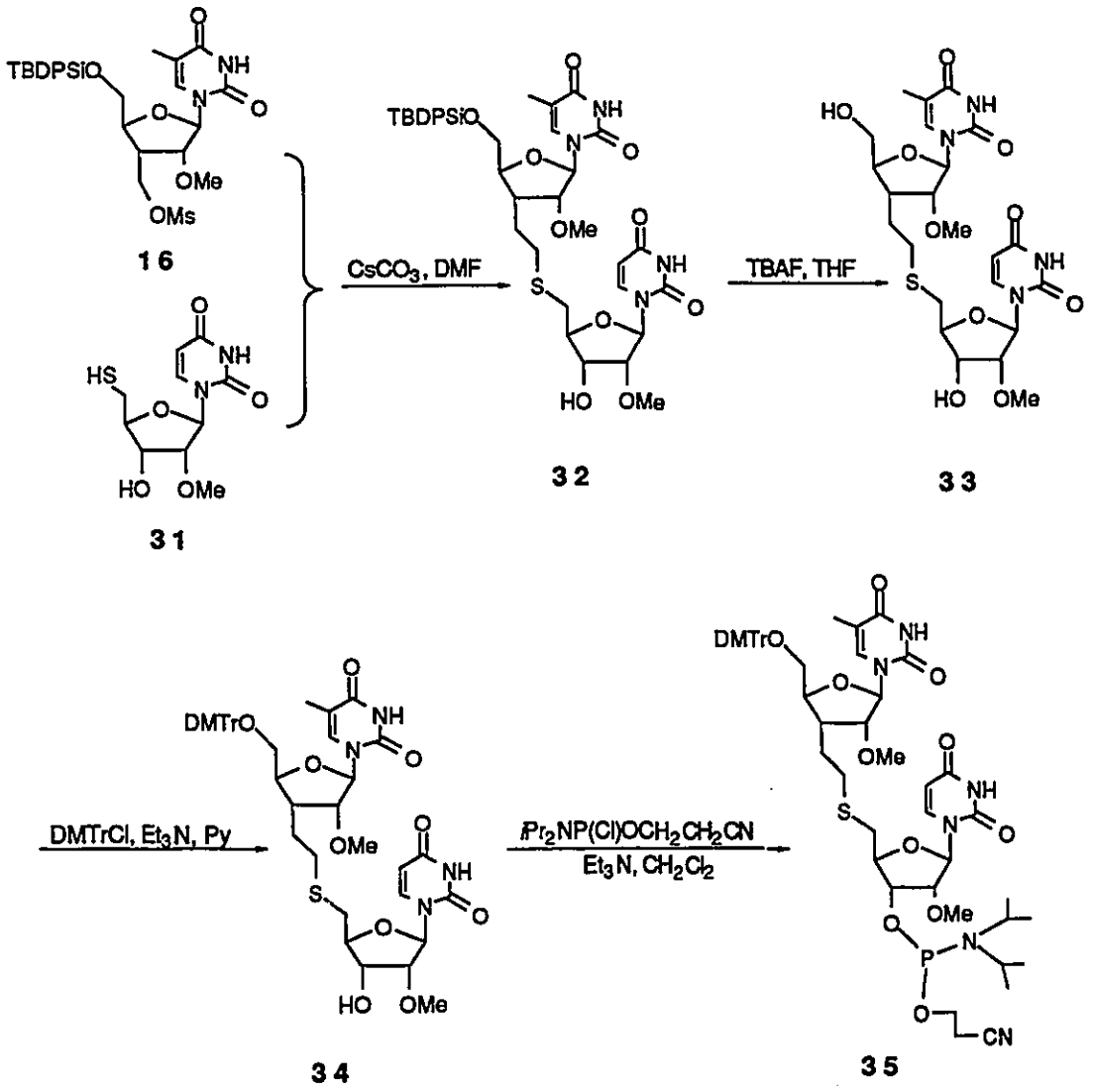


Figure 20 The 500 MHz ^{1}H-NMR spectrum of diol **33** in H_2O.

Scheme 23


TBDPSiO
OMe
OMs
16
HS
HO
OMe
31
$CsCO_3$, DMF
32
TBAF, THF
HO
33
DMTrO
DMTrCl, Et_3N, Py
34
$iPr_2NP(Cl)OCH_2CH_2CN$
Et_3N, CH_2Cl_2
CN
35

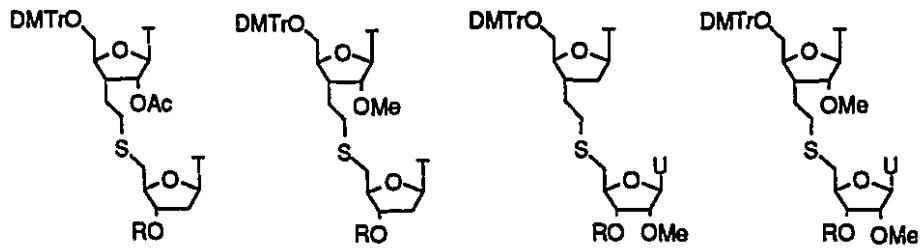
Chapter 5. Preparation and Binding Properties of Oligonucleotides Containing Sulfide-linked Dimers

5.1 Preparation of Sulfide-linked Oligonucleotides

For comparison purposes, the sequence chosen for incorporation of the modified dinucleoside $GCGT_6GCT$ was identical to that used by Kawai and Wang[52].

The modified DNA dodecamers **36-47** were synthesized by the phosphoramidite method[89] using standard solid-support techniques (Figure 21). Four different sulfide dimers were incorporated into DNA. They were the sulfide-linked ribofuranosylthymine-thymidine dimer **9** ($T^{CH}_{s}T$) (Scheme 24), O^2-methylribofuranosylthymine-thymidine **21** ($T^{OMe}_{s}T$), thymidine-O^2-methyluridine **A** (TU^{OMe}_{s})[90] and O^2-methylribofuranosylthymine-O^2-methyluridine **35** ($T^{OMe}_{s}U^{OMe}_{s}$). In order to investigate the effects of the incorporation of sulfide modification in DNA, the number of sulfide dimers being incorporated varied from 1-3.

Scheme 24



9 **21** **A** **35**

R = i-$Pr_2N(CNCH_2CH_2O)P$

89 Caruthers, M. H.: *Science*, **230**, 281 (1985).

90 This dimer was first prepared by Dr. D, Wang.

36: 5'- d-G C G T T T T^{OH}_{s} T T T G C T -3'

37: 5'- d-G C G T^{OH}_{s} T T T T T^{OH}_{s} T G C T -3'

38: 5'- d-G C G T^{OH}_{s} T T^{OH}_{s} T T^{OH}_{s} T T G C T -3'

39: 5'- d-G C G T T T T^{OMe}_{s} T T T G C T -3'

40: 5'- d-G C G T^{OMe}_{s} T T T T T^{OMe}_{s} T G C T -3'

41: 5'- d-G C G T^{OMe}_{s} T T^{OMe}_{s} T T^{OMe}_{s} T G C T -3'

42: 5'- d-G C G T T $T_{s}U^{OMe}$ T T G C T -3'

43: 5'- d-G C G $T_{s}U^{OMe}$ T T $T_{s}U^{OMe}$ G C T -3'

44: 5'- d-G C G $T_{s}U^{OMe}$ $T_{s}U^{OMe}$ $T_{s}U^{OMe}$ G C T -3'

45: 5'- d-G C G T T T T^{OMe}_{s} U^{OMe} T T G C T -3'

46: 5'- d-G C G $T^{OMe}_{s}U^{OMe}$ T T T $T^{OMe}_{s}U^{OMe}$ G C T -3'

47: 5'- d-G C G $T^{OMe}_{s}U^{OMe}$ $T^{OMe}_{s}U^{OMe}$ $T^{OMe}_{s}U^{OMe}$ G C T -3'

48: 5'- d-G C G T T T T T T G C T -3' (DNA)

49: 3'- d-C G C A A A A A A C G A -5' (DNA)

50: 3'- r-C G C A A A A A A C G A -5' (RNA)

Figure 21 Sequences of oligomers **36-48** are shown in the 5' to 3' direction, and complementary DNA **49** and RNA **50** are shown in the 3' to 5' direction. $T^{OH}_{s}T$ denotes the ^{5}2'-OH sulfide-linked dimer (**9**); $T^{OMe}_{s}T$ represents the ^{5}2'-OMe sulfide-linked dimer (**21**); $T_{s}U^{OMe}$ stands for the ^{3}2'-OMe sulfide linked dimer (**A**), and $T^{OMe}_{s}U^{OMe}$ for the di-^{5,3}2'-OMe sulfide-linked dimer (**35**).

All purine oligonucleosides were protected with ammonia labile benzoyl groups. A fifth bottle containing a 0.11 M solution of the modified sulfide dimers **9**, **21**, **A** or **35** in acetonitrile, was attached to the DNA synthesizer. All sequences were prepared in the "trityl off" mode and on a 0.2 μmole scale. The reaction time for the tetrazole-mediated coupling of **9**, **21**, **A** or **35** to the growing chain was increased to 9 minutes from the standard 2 min. The fully natural DNA strands **48** and **49** were also prepared by standard solid-phase methods. We thank Dr. Y. Sanghvi, ISIS, Pharmaceuticals, for making available to us RNA dodecamer **50**.

The average coupling efficiencies of the oligomers **36-49** were automatically measured by the synthesizer, and were 78-100%. The oligomers were deprotected and cleaved from the solid-support by treating an oligo-support cassette with a 29% ammonia solution at 55°C for 16 hours. Then, the solution of the oligomer in aqueous ammonia was desalted using a Sephadex NAP-10 column. The single absorption band observed on polyacrylamide gel for all oligomers indicated their high purity. Based on the analysis of mass to charge ratios of the oligomers prepared, **48** and **49** should move fastest on the gel since they have lowest mass/charge ratios; on the other hand, oligomers containing sulfide linkages should have increased mass/charge ratios and therefore retarded mobility relative to **48** and **49**. The more alkyl sulfide dimers were incorporated, the slower they moved on the gel. The results demonstrated on the gel were in agreement with the expectations.

5.2 Preliminary Binding Properties of Complexes 38/49 and 38/50

The binding properties of modified oligomer **38** which contained three sulfide-linked dimers $T^{CH}_{2}T$ was initially investigated. The results of thermal denaturation studies are summarized in Table 3. The data clearly indicated that the strands containing $T^{CH}_{2}T$ dimers can discriminate between complementary DNA and RNA over a wide range of salt concentrations. As expected, cooperative binding between unmodified DNA strands **48** and **49**, and between **48** and natural RNA oligomer **50**, was observed with melting temperatures (*Tm*) of 55°C and 52°C (100 mM NaCl), respectively. In the case of hybridization between the sulfide-containing oligomer **38** and RNA **50**, a melting temperature of 34°C was measured at the same salt concentration. This corresponded to a decrease in *Tm* of 6.3°C per sulfide-substitution and indicated that, while weakening the complexation to targeted RNA, the modification still allowed for cooperative binding. However, in the case of complexation between modified oligomer **38** and DNA dodecamer **49**, no cooperative transition was observed over a wide range of salt concentrations.

Table 3 Melting temperatures *Tm* [°C].

c (NaCl) [mM]	48/49	48/50	38/49	38/50
10	47		[b]	19
100	55	52	[b]	34
210	58		[b]	39
910	65		[b]	40

48: wild type DNA dodecamer

49: complementary DNA dodecamer

50: complementary RNA dodecamer

[b] No observable Tm.

This selectivity is also clearly demonstrated by native-gel hybridization. Mixtures of the complementary oligomers **38** and DNA **49**, **38** and RNA **50**, DNA **48** and DNA **49**, and DNA **48** and RNA **50** were incubated in 30% sucrose solutions containing 10 mM magnesium chloride and then applied to separate lanes of a polyacrylamide gel containing no urea. Oligomers **38**, **48**, **49** and **50** were also applied individually. After electrophoresis the gel showed bands corresponding to duplexes DNA **48**/DNA **49** and DNA **48**/RNA **50**, as well as that formed between **38** and RNA **50**. Moreover, this latter band migrated much more slowly than either **48/49** or **48/50**, reflecting the lower charge (three fewer phosphate groups) of the complex **38/50**. In contrast, the lane containing the mixture of **38** and DNA **49** showed only the two bands corresponding to the unassociated single strands.

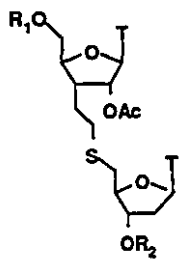
This selectivity exhibited by **38** for RNA is quite surprising, and therefore the oligomers containing one or two sulfide-dimers were synthesized for further understanding of binding properties of sulfide-modified backbone of oligomers.

5.3 Thermal Denaturations

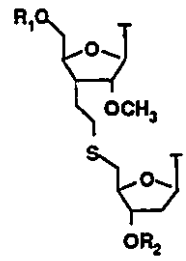
The thermal stabilities of duplexes formed between oligomers **36-48** and a complementary DNA, d-5'-AGCAAAAAACGC (**49**), or RNA target, r-5'-AGCAAAAAACGC (**50**), were studied. *Tm* values for the formed duplex association are listed in Table 4 and representative thermal denaturation curves are shown in Figure 22-25.

Cooperative binding between natural DNA **48** and **49**, and between **48** and RNA **50**, were observed with melting temperature of 59.5°C and 52.5°C, respectively in a 1.0 M NaCl buffer. In the cases of the hybridizations between oligomer **36** and DNA **49**, or RNA **50**, and between **37** and **49**, or **50**, the *Tm* values indicated that, while weakening the complexation to targets DNA and RNA, the modification still allowed for good cooperative binding. The melting temperature of these oligomers **36** and **37** were decreased by about 14°C per sulfide replacement for target DNA, and about 7.5°C for target RNA. However, oligomer **38**, in which three hydroxy sulfide dimers were incorporated, demonstrated binding ability only to its complementary RNA, but not to its complementary DNA. Those reductions of *Tm* may be related to the introduction of mixed ribo-deoxyribo modifications, where the 2'-hydroxyl of the ribo-sugar may adopt a 3'-endo conformation. It is well known that DNA/DNA duplexes adopts B-form in which the sugars have 2'-endo conformation. Thus, the incorporations of the modified sulfide dimer may discontinue the B-form of those 12-mers and make the *Tm*'s lower than parent ones. In addition, the steric interactions between the 5'-sulfur and either 2'-hydroxyl or the H6 of pyrimidine, specially the former, may be another major factor to cause the decrease of the binding properties of the oligomers containing sulfide dimers[46,47].

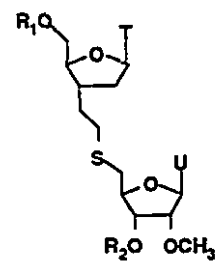
Table 4 Melting temperatures of the duplexes formed between sulfide-linked oligomers and their complementary DNA and RNA.



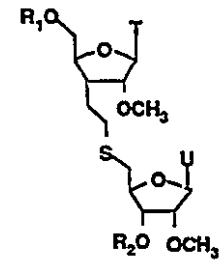
$T^{OH}_{s}T$



$T^{OMe}_{s}T$



$T_{s}U^{OMe}$



$T^{OMe}_{s}U^{OMe}$

Entry	Sequence	Tm DNA (49)	Tm RNA (50)
48	5'-GCGTTTTTTGCT -3'	59.5	52.5
36	5'-GCGTTT$^{OH}_{s}$TTTGCT -3'	45.1	45.2
37	5'-GCGT$^{OH}_{s}$TTTT$^{OH}_{s}$TGCT -3'	31.3	37.5
38	5'-GCGT$^{OH}_{s}$TT$^{OH}_{s}$TT$^{OH}_{s}$TGCT -3'	N*	31.5
39	5'-GCGTTT$^{OMe}_{s}$TTTGCT -3'	44.0	44.0
40	5'-GCGT$^{OMe}_{s}$TTTT$^{OMe}_{s}$TGCT -3'	30.1	36.0
41	5'-GCGT$^{OMe}_{s}$TT$^{OMe}_{s}$TT$^{OMe}_{s}$TGCT -3'	N*	30.5
42	5'-GCGTTT$_{s}$U^{OMe}TTGCT -3'	47.8	45.7
43	5'-GCGT$_{s}$U^{OMe}TTT$_{s}$U^{OMe}GCT -3'	37.7	40.8
44	5'-GCGT$_{s}$U^{OMe}T$_{s}$U^{OMe}T$_{s}$U^{OMe}GCT -3'	26.2	35.5
45	5'-GCGTTT$^{OMe}_{s}$U^{OMe}TTGCT -3'	45.1	47.5
46	5'-GCGT$^{OMe}_{s}$U^{OMe}TTT$^{OMe}_{s}$U^{OMe}GCT -3'	32.9	45.5
47	5'-GCGT$^{OMe}_{s}$U^{OMe}T$^{OMe}_{s}$UT$^{OMe}_{s}$U^{OMe}GCT-3'	24.8	43.8

* N = no binding.

Oligomers **39**, **40** and **41**, in which 1 to 3 methoxy sulfide dimers were incorporated, respectively, had almost the same binding ability as the corresponding hydroxy sulfide-containing oligomers.

Oligomers **42**, **43** and **44**, which also contained methoxy sulfide dimers, but at 3'-end position, showed higher melting temperatures than those of oligomers **39-41** in which the methoxy group was positioned at 5'-end. For the hybridization between **42-44** with their complementary RNA, a decrease in *Tm* of per sulfide-substitution was 5.7°C, while a decrease of 7.3°C per sulfide-substitution was observed for **39-41**. This observation supports the assumption that the steric interaction between the sulfur atom and 2'-substitution is one of the facts responsible for the reduction of binding.

The *Tm* measurement of dimethoxy-sulfide containing oligomers **45-47** showed very interesting results. For these oligomers, the decrease of per sulfide-substitution was 11.1°C for complementary DNA, but only 2.9°C for complementary RNA.

Figures 26-30 are circular dichroism spectra of hybrids of **42-48** with their complementary DNA or RNA, from which some information about the conformation change between unmodified and modified duplexes may be deduced. According to the report of Lesnik[35] *et al*, the maximum of the positive band in the CD spectrum of the DNA:RNA hybrid is at 270 nm, between that of the DNA:DNA and RNA:RNA maxima, and, at wavelengths below 250 nm (negative band), the ellipticity of the DNA:RNA hybrid is also between those of the DNA:DNA and RNA:RNA duplexes. These observations suggested that the geometry of the DNA:RNA hybrid is an intermediate between the B-form and the A-form. In our cases, the CD of **48/50** showed a maximum lower than that of **48/49** (Figure 26) at either

positive or negative band, and therefore indicated that its conformation was changed from a B-form to an A-B intermediate.

CD-Spectra of the hybrids of **45**, **46** and **47** with their complementary DNA **49** also exhibited a maximum lower than that of **48/49** at either positive or negative band (Figure 28), while the change of maxima of **42**, **43** and **44** with their complementary DNA only demonstrated at positive band (Figure 27). Similar CD-spectra results were observed in the hybrids between **45**, **46** and **47** with their complementary RNA (Figure 30), and between **42**, **43** and **44** with their complementary RNA (Figure 29, the change occurred at negative band). These observations suggest that conformation of 2'-modification hybrids may be more like A-form than the natural DNA, especially for the dimethoxy-sulfide-linked oligomers **45**, **46** and **47**, and therefore explain the interesting binding results found in the duplexes **45/50**, **46/50** and **47/50**.

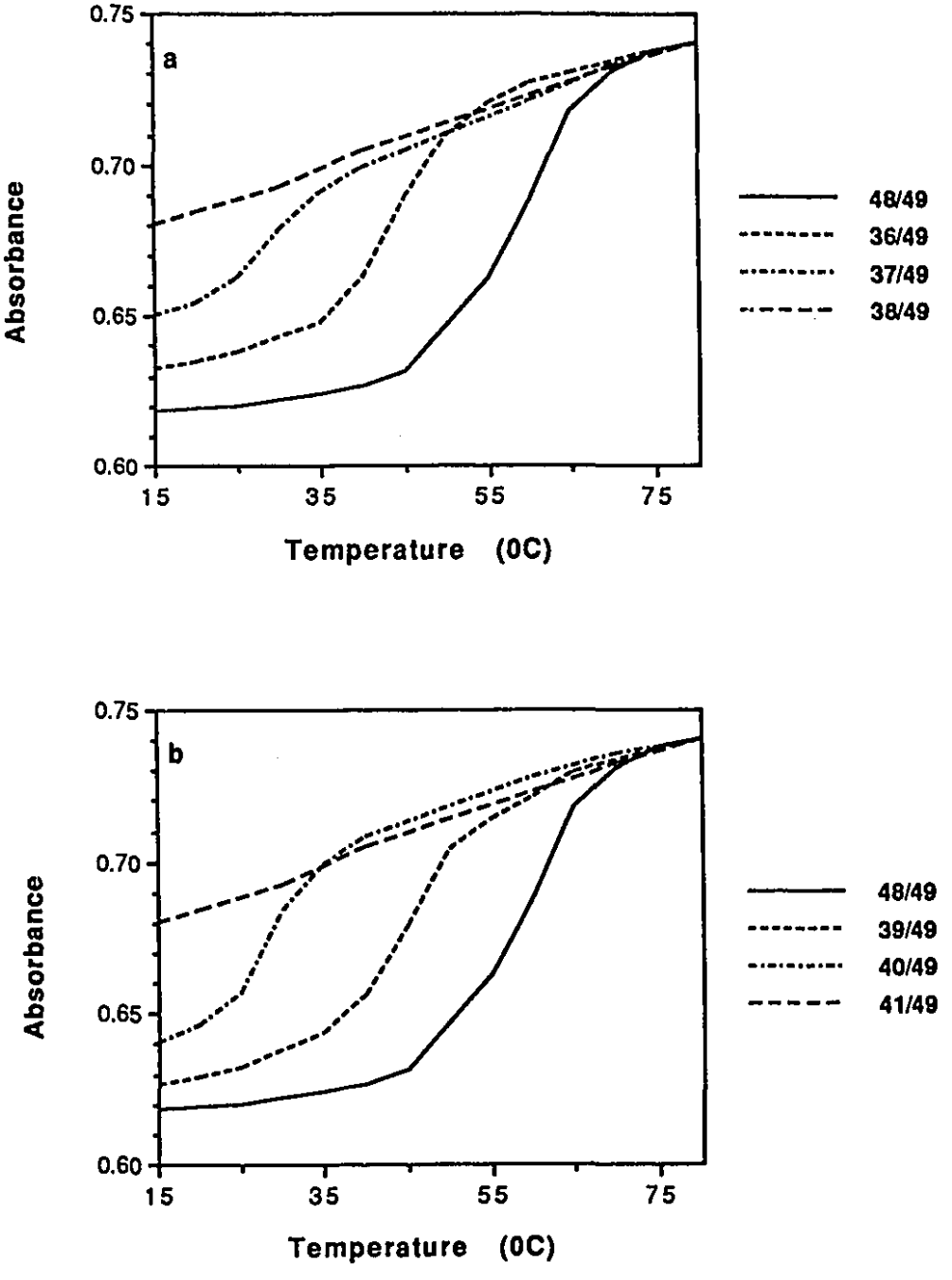


Figure 22 Thermal denaturation curves. a) Melt curves for complexes **48/49, 36/49, 37/49** and **38/49.** b) Melt curves for complexes **48/49, 39/49, 40/49** and **41/49.**

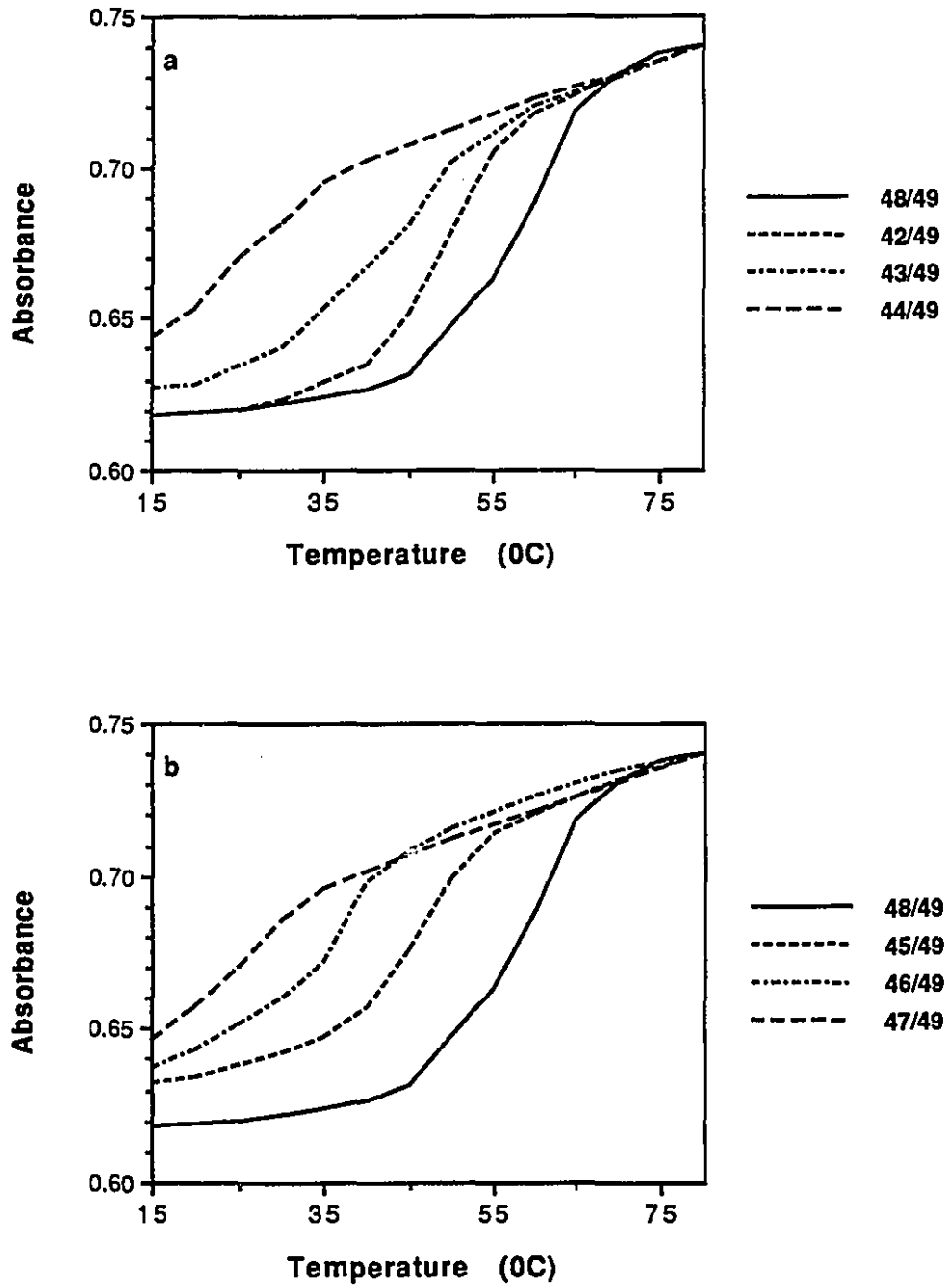


Figure 23 Thermal denaturation curves. a) Melt curves for complexes **48/49**, **42/49**, **43/49** and **44/49**. b) Melt curves for complexes **48/49**, **45/49**, **46/49** and **47/49**.

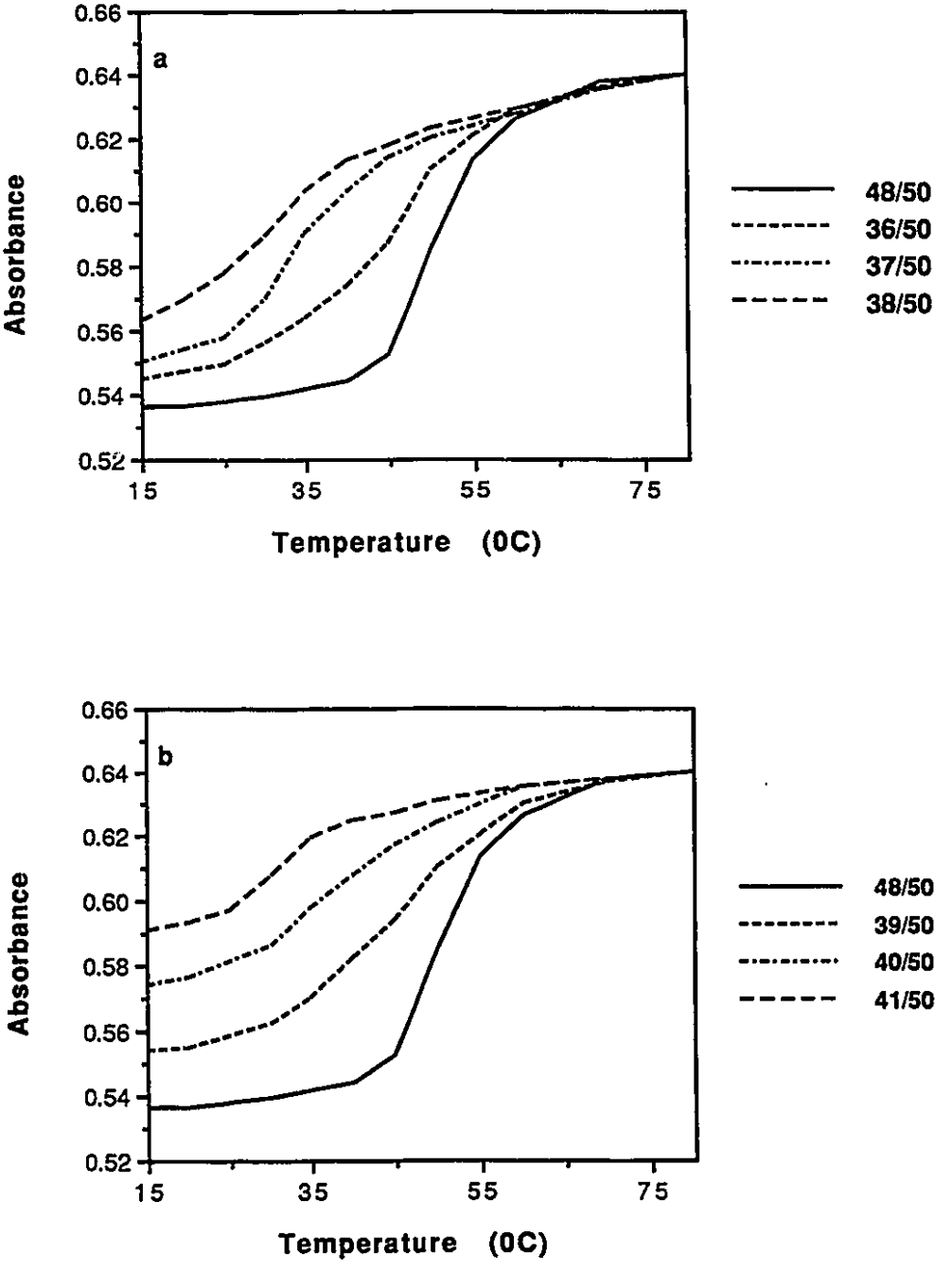
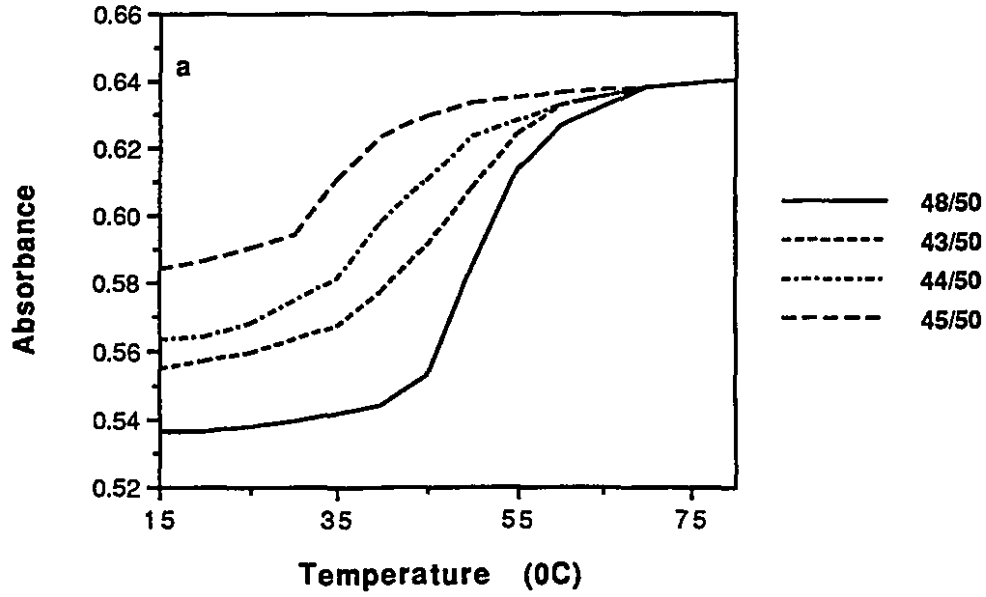


Figure 24 Thermal denaturation curves. a) Melt curves for complexes **48/50, 36/50, 37/50** and **38/50.** b) Melt curves for complexes **48/50, 39/50, 40/50** and **41/50.**



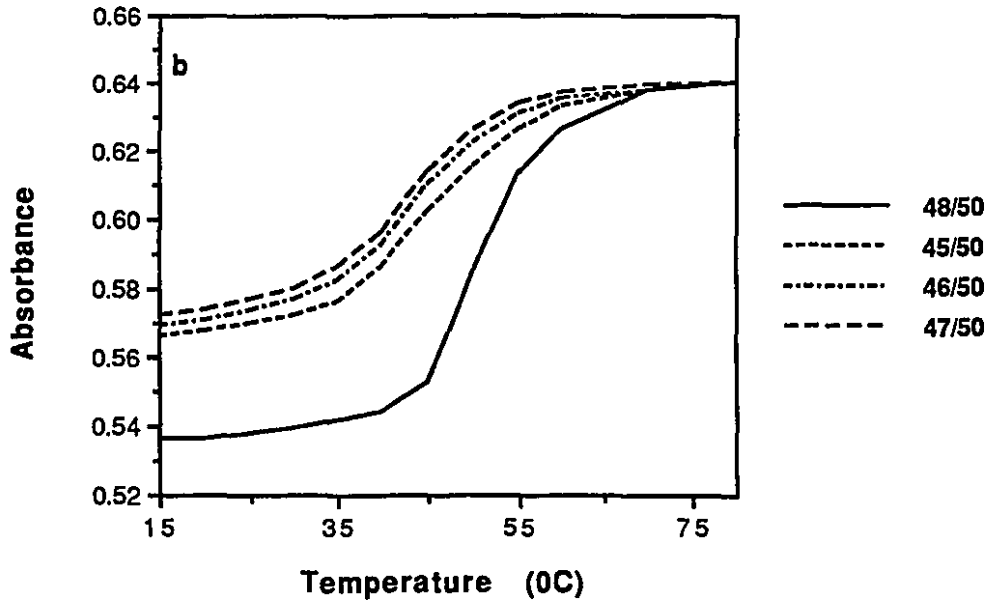


Figure 25 Thermal denaturation curves. a) Melt curves for complexes **48/50, 42/50, 43/50** and **44/50.** b) Melt curves for complexes **48/50, 45/50, 46/50** and **47/50.**

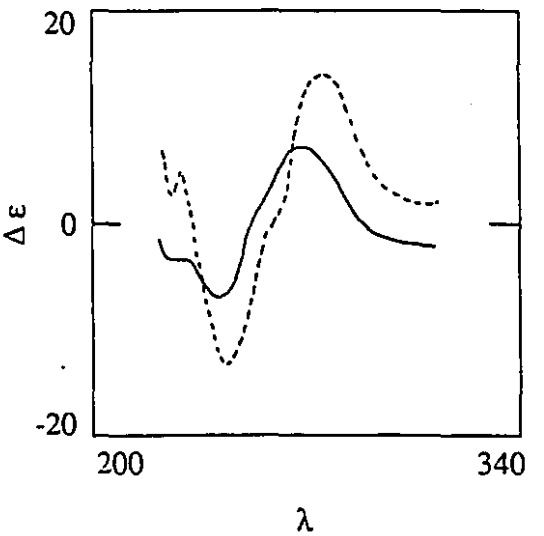


Figure 26 CD of duplexes of **48/49** (·····) and **48/50** (———).

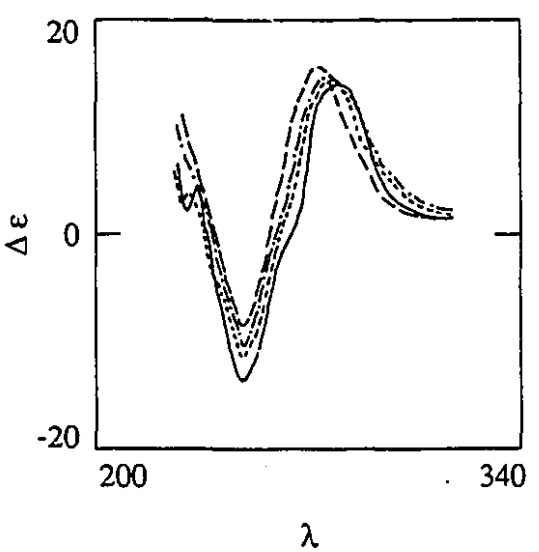


Figure 27 CD of duplexes of **48/49** (———), **42/49** (·····), **43/49** (—·—) and **44/49** (— —).

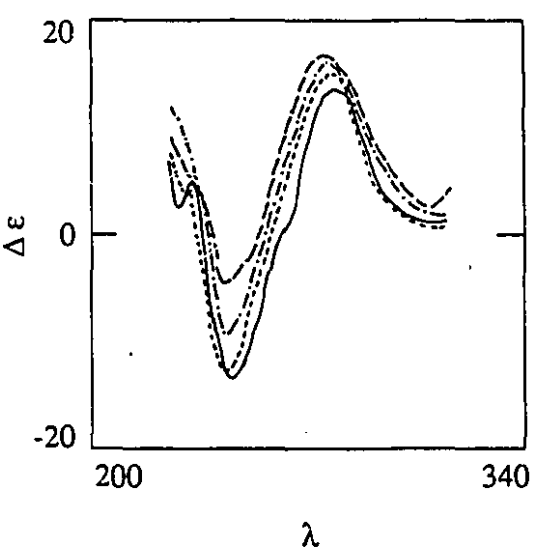


Figure 28 CD of duplexes of **48/49** (———), **45/49** (·····), **46/49** (—·—) and **47/49** (— —).

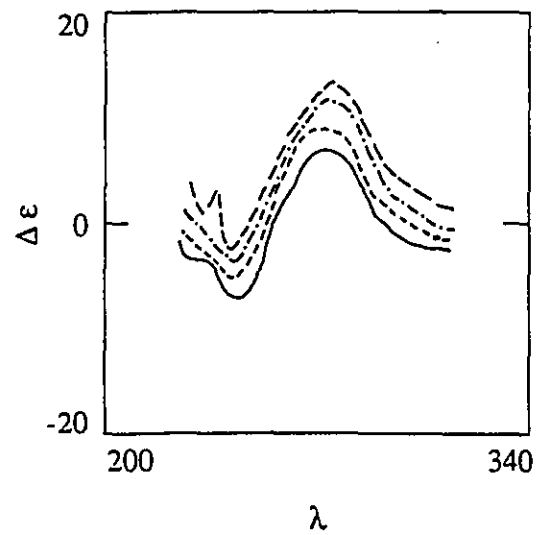


Figure 29 CD of duplexes of **48/50** (———), **42/50** (·····), **43/50** (— · —) and **44/50** (— —).

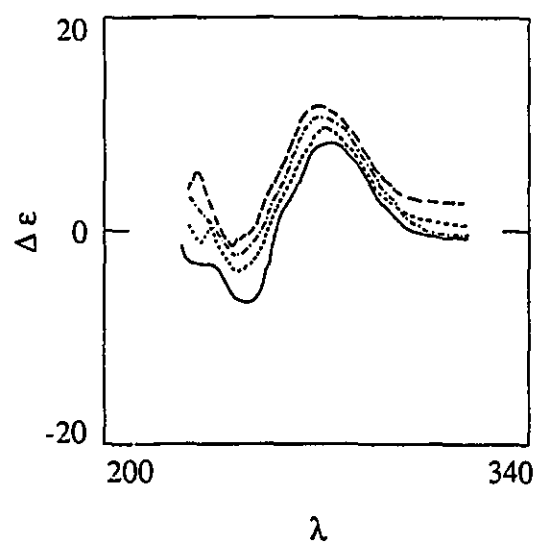
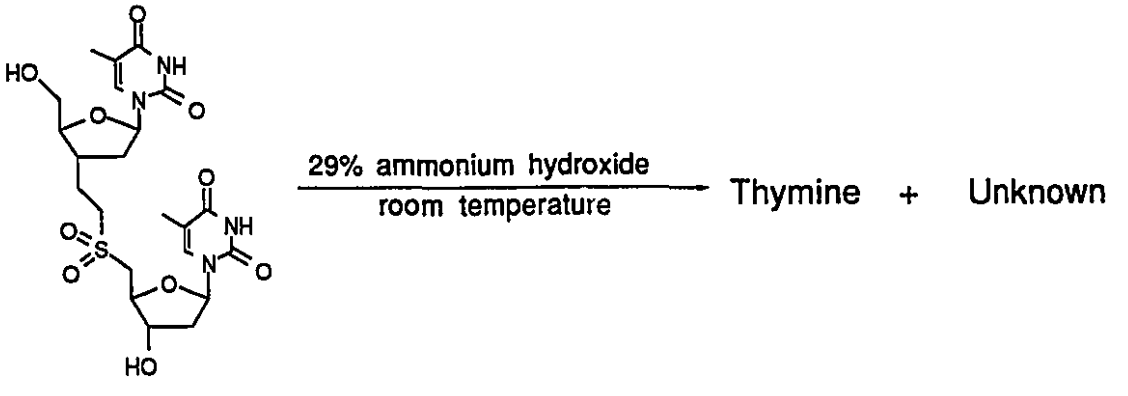


Figure 30 CD of duplexes of **48/50** (———), **45/50** (·····), **46/50** (— · —) and **47/50** (— —).

Chapter 6 Synthesis and Binding Properties of Sulfone-containing Oligonucleotides

Oligomers containing sulfone backbones should have quite different binding properties than the sulfide-linked oligomers, and therefore it is of great interest to investigate their syntheses and binding properties. As described by Wang[77], the synthesis of oligonucleotide, with three incorporated sulfone dimer **S1** (page 28), was unsuccessful. The lack of success was attributed to β-elimination, resulting in the decomposition of the sequence during the cleavage from the solid-support using 29% ammonium hydroxide, as proven by model studies (Scheme 25). In this study, it was therefore decided to carry out the oxidation of the sulfide-containing oligomers to the sulfones after the ammonia treatment necessary to remove the protecting groups.

Scheme 25



The solution of a sulfide-containing oligomer was treated with aqueous oxone stock (pH 6.4, phosphate buffer) at room temperature for 16

h[91,92]; then the oligomer was desalted on a Sephadex NAP-10 column, and the collected eluate was quantified at 260 nm. The expected sulfone-containing sequences **51-62** are listed in Table 5.

Electrophoresis showed no distinguishing difference between oxidized and unoxidized samples. To identify the formation of sulfone-backbones, 0.3 OD (optical density) units of **53** was treated with 29% ammonium hydroxide at 55°C for 2 h. Then this sample was applied to a PAGE gel and no UV absorption band was observed on the gel. This observation lead to the conclusion that the treatment with NH_3 decomposed sample **53** to smaller pieces than 10-mer; in the other words, **53** was sulfone-containing oligomer.

Tm values of sulfone-containing dodecamers are also listed in Table 5. The binding properties for sulfone-containing oligomers were all much poorer than those of corresponding sulfide-containing oligomers. Except for **51**, **54**, **57** and **60**, there was no binding between modified oligomers and their complementary DNA. Hybrids of sulfone-containing oligomers with their complementary RNA were considerably more stable than hybrids with complementary DNA, except **55-57**. The smallest ΔTm of 8.8°C was measured in the hybridization of dimethoxy-sulfone-linked oligomers, which was consistent with the results observed in that of dimethoxy-sulfide-linked oligomers.

The poorer binding ability of sulfone oligomers, compared to sulfide oligomers was probably due to the size of sulfone group, which is bigger than that of the sulfide group. It therefore resulted in more steric interaction with the neighbouring groups, thereby reducing the *Tm* values.

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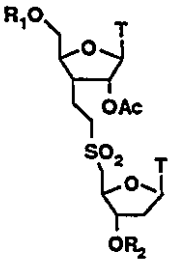
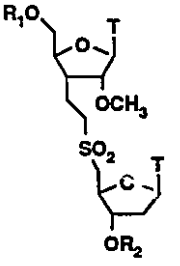
91 Murray, R. W.; Jeyaraman, R.: *J. Org. Chem.*, **50**, 2847 (1985).

92 Trost, B. M.; Curran, D. P.: *Tetrahedron Lett.*, **22**, 1287 (1981).

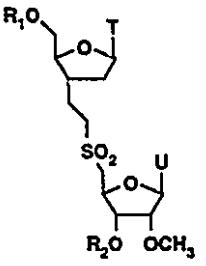
Table 5 Melting temperatures of the duplexes formed between sulfone-linked oligomers and their complementary DNA and RNA.



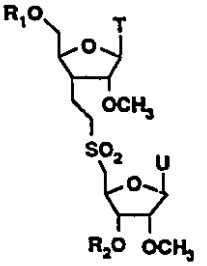
$T^{OH}_{SO_2}T$



$T^{OMe}_{SO_2}T$



$T_{SO_2}U^{OMe}$



$T^{OMe}_{SO_2}U^{OMe}$

Entry	Sequence	Tm	
		DNA (49)	RNA (50)
48	5'-GCGTTTTTTGCT -3'	59.5	52.5
51	5'-GCGTTT$^{OH}_{SO_2}$TTTGCT -3'	35.0	35.9
52	5'-GCGT$^{OH}_{SO_2}$TTTT$^{OH}_{SO_2}$TGCT -3'	~N*	25.5
53	5'-GCGT$^{OH}_{SO_2}$TT$^{OH}_{SO_2}$TT$^{OH}_{SO_2}$TGCT -3'	N*	< 20
54	5'-GCGTTT$^{OMe}_{SO_2}$TTTGCT -3'	34.9	34.8
55	5'-GCGT$^{OMe}_{SO_2}$TTTT$^{OMe}_{SO_2}$TGCT -3'	N*	N*
56	5'-GCGT$^{OMe}_{SO_2}$TT$^{OMe}_{SO_2}$TT$^{OMe}_{SO_2}$TGCT -3'	N*	N*
57	5'-GCGTTT$_{SO_2}$U^{OMe}TTGCT -3'	34.8	38.7
58	5'-GCGT$_{SO_2}$U^{OMe}TTT$_{SO_2}$U^{OMe}GCT -3'	~N*	28.6
59	5'-GCGT$_{SO_2}$U^{OMe}T$_{SO_2}$U^{OMe}T$_{SO_2}$U^{OMe}GC -3'	N*	< 22
60	5'-GCGTTT$^{OMe}_{SO_2}$U^{OMe}TTGCT -3'	35.9	35.9
61	5'-GCGT$^{OMe}_{SO_2}$U^{OMe}TTT$^{OMe}_{SO_2}$U^{OMe}GCT -3'	~N*	30.4
62	5'-GCGT$^{OMe}_{SO_2}$U^{OMe}T$^{OMe}_{SO_2}$U^{OMe}T$^{OMe}_{SO_2}$U^{OMe}GCT-3'	N*	25.0

* N = no binding

An attempt was made to characterize the sulfone-containg oligomers by electrospray mass spectrometry. Unfortunately, due to our inability to remove all the salts, and due to the very small amount of oligomers available (0.15 OD-0.3 OD units), no positive identification of the oligomer could be made.

Contribution to Knowledge

1. Three 2'-modified sulfide-linked dimers have been synthesized.

2. Four 2'-modified sulfide-linked dimers have been incorporated into a 12-mer DNA strand, individually, by automated technology. The number of modified dimers incorporated varied from 1-3. The binding properties of those oligonucleotides with their complementary DNA and RNA were investigated.

3. Sulfone-containing oligomers were obtained by the oxidation of sulfide-containing oligomers, and their binding properties were also measured.

EXPERIMENTAL

General Methods.

Melting points (m.p.) were determined using an Electrothermal MP apparatus and are uncorrected. Low-resolution chemical ionization mass spectra (CI) were obtained on an Hp 5980A quadrupole mass spectrometer in the direct-inlet mode. High-resolution CI, low-resolution and high-resolution FAB mass spectra (HRMS) were obtained on a VG ZAB-HS sector mass spectrometer, in the direct-inlet mode. The measurements were generally carried out at a resolving power (res.) of 10000 unless otherwise indicated.

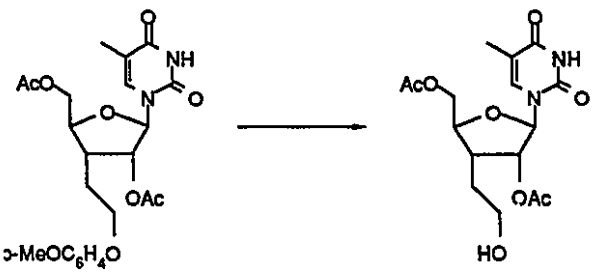
^{1}H-NMR spectra were recorded on either Varian XL200 or Varian XL300 spectrometer and the assignments are based on homonuclear decoupling and / or COSY experiments. The stereochemistry was confirmed base on COSY and nOe experiments. When deuteriochloroform was employed as solvent, internal tetramethylsilane (TMS) was used as the reference. The residual proton signal methanol, assigned a value of 3.30 ppm, was used as reference in this case. The multiplicities are recorded using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; m^{n}, symmetical signal of n lines; br, broad. ^{13}C-NMR spectra were obtained also using a Varian XL 200 or XL 300 spectrometer, at 55.5 MHz and 75.4 MHz. The $^{13}CDCl_3$, $^{13}CD_3OD$ and $^{13}CD_3COCD_3$ signals, assigned values of δ 77.00, 49.00 and 30.00 respectively, were used as references in these solvents. Peak assignments were, in some cases, made with the aid of APT experiment. ^{31}P-NMR were obtained on XL 200 at 81.0 MHz with aqueous phosphoric acid as exterior reference. Preceding superscripts in the parentheses of the NMR data indicated to which

branched-chain nucleoside unit the proton or carbon belonged (5 as 5'end and 3 as 3'-end).

Tetrahydrofuran was distilled from sodium benzophenone ketyl. Methylene chloride and 1,2-dichloroethane were distilled from P_2O_5. Toluene was dried over sodium wire. Triethylamine, acetonitrile and pyridine were distilled from calcium hydride. *N,N*-Dimethylformamide was dried by shaking with KOH followed by distillation, at reduced pressure, from BaO. Thin-layer chromatography (t.l.c.) was performed using Kieselgel 60 F_{254} aluminium-backed plates (0.2 mm thickness) and visualized by UV and / or dipping in a solution of ammonium molybdate (2.5 g) and ceric sulfate (1 g) in 10 % v/v aqueous sulphuric acid (100 mL), followed by heating. Kieselgel 60 (Merck 230-400 mesh) silica gel was employed for column chromatography. The ratio of silica gel to substance to be purified is approximately 20 : 1.

Experimental for Chapter 2

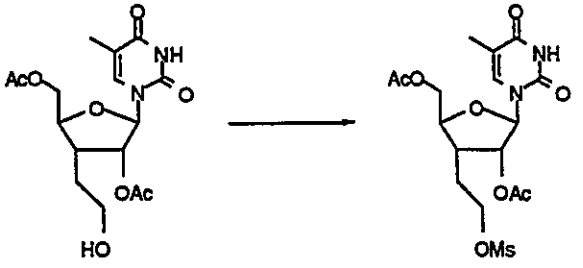
Alcohol 2



Ceric ammonium nitrate (11.85 g, 21.6 mmole) was added to a solution of **1** (4.82 g, 10.1 mmole) in acetonitrile-water (1:1, 56 ml) at 0°C. The reaction was stirred at 0°C for 30 min and diluted with brine (116 ml). The mixture was extracted with ethyl acetate (3 x 200 ml). The organic extracts were washed with sodium sulfite (10% w/v, until the aqueous layer remained colorless), sodium bicarbonate (5% w/v, 100 ml), and dried (Na_2SO_4). Removal of the solvent yielded a yellow foam which was chromatographyed over silica gel (20:1 CH_2Cl_2 / MeOH, v/v) and afforded the alcohol **2** (2.83 g, 76%), m.p. 126-127°C (EtOH / H_2O): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.78-1.88 (m, 2H, H1"), 1.93 (s, 3H, 5-Me), 2.07 and 2.09 (2s, 6H, OAc), 2.55 (h^7, 1H, H3'), 3.68 (t, 2H, H2"), 4.19 (ddd, 1H, H4'), 4.42 (d, 2H, $H5'_{AB}$), 5.53 (d, 1H, H2'), 5.56 (s, 1H, H1'), 7.31 (s, 1H, H6), 9.2 (br s, 1H, NH), $J_{H1'-H2'}$ = 1.2, $J_{H2'-H3'}$ = 6.0, $J_{H3'-H4'}$ = 10.5, $J_{H4'-H5'A}$ = 3.5 , $J_{H4'-H5'B}$ = 2.5, $^2J_{H5'A-H5'B}$ = -12.8; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 13.50 ppm (5-Me), 21.25, 21.45 (COMe), 29.20 (C1"), 40.11 (C3'), 61.33 (C5'), 64.92 (C2"), 79.28 (C2'), 84.34 (C4'), 93.13 (C1'), 112.00 (C5), 138.61 (C6), 152.58 (C2),

163.85 (C4), 172.11 and 172.94 (COMe); MS (FAB - NBA) m/e 371 ([MH^+], 46%), 311 ([MH^+ - AcOH], 7.2), 245 ([MH^+ - ThyH], 100).

Mesylate 3

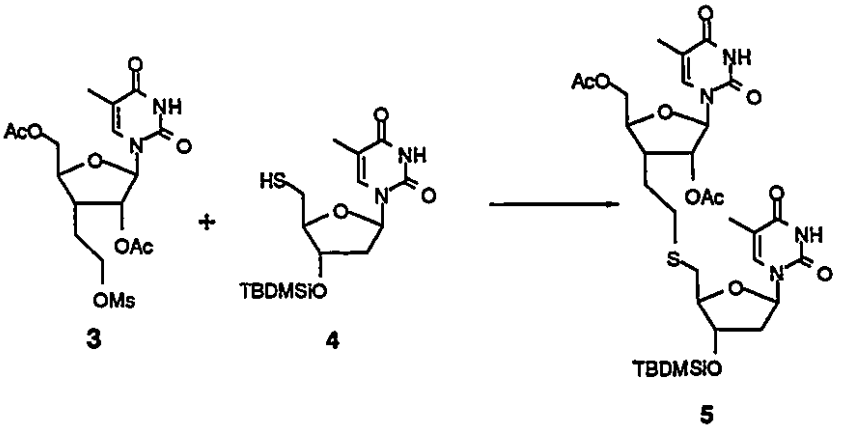


2 **3**

Methanesulfonyl chloride (0.48 ml, 6.20 mmole) was added to a stirred solution of alcohol **2** (1.00 g, 2.70 mmole) and dry triethylamine (0.68 ml, 4.88 mmole) in dry dichloromethane (19 ml) at room temperature under nitrogen. After 1 h, the reaction was diluted with dichloromethane (20 ml) and washed with hydrochloric acid (5% w/v, 7 ml), saturated aqueous sodium bicarbonate (7 ml), and brine (5% w/v, 7 ml). The organic layer was dried (Na_2SO_4), and the solvent was removed yielding a yellow foam. Chromatography over silica gel (20:1 CH_2Cl_2 / MeOH, v/v) afforded the mesylate **3** as a white foam (1.17 g, 97%): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.72-1.98 (m, 2H, H1"), 1.93 (s, 3H, 5-Me), 2.13 and 2.16 (two s, 6H, OAc), 2.55 (h^7, 1H, H3'), 3.02 (s, 3H, SO_2Me), 4.12 (dq, 1H, H4'), 4.24-4.29 (m, 2H, H2"), 4.37 (A of ABX, 1H, $H5'_A$), 4.40 (B of ABX, 1H, $H5'_B$), 5.51 (dd, 1H, H2'), 5.64 (d, 1H, H1'), 7.23 (s, 1H, H6), 9.05(br s, 1H, NH), $J_{H1'-H2'}$ = 1.3, $J_{H2'-H3'}$ = 5.9, $J_{H3'-H4'}$ = 19.3, $J_{H4'-H5'A}$ = 4.6, $J_{H4'-H5'B}$ = 2.5, $^2J_{H5'A-H5'B}$ = -10.2; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.51 ppm (5-Me), 20.57 (2 x COMe), 24.25 (C1"),

37.23 (SO_2Me), 38.34 (C3'), 62.94 (C5'), 67.98 (C2"), 77.00 (C2'), 81.784 (C4'), 91.77 (C1'), 110.72 (C5), 136.39 (C6), 150.31 (C2), 164.28 (C4), 160.99, 170.62 (COMe); MS (FAB-NBA) m/e 449 ([MH^+], 46 %), 389 ([MH^+ - AcOH], 5.7), 323 ([MH^+ - ThyH], 100); HRMS (FAB - NBA) m/e calcd. for $C_{17}H_{24}N_2O_{10}$ + H^+: 449.12300, found: 449.12316.

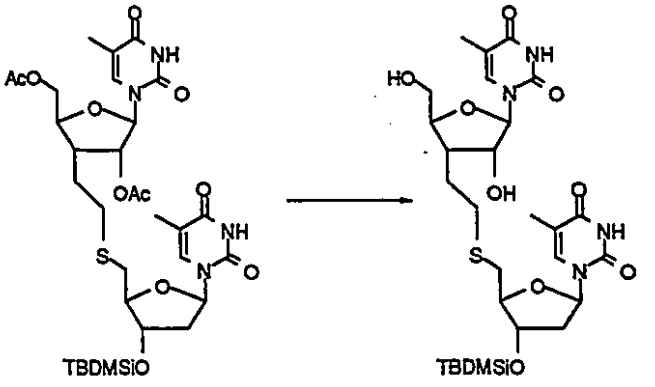
Dimer 5



Cesium carbonate (549 mg, 1.68 mmol) was suspended in dry, deoxygenated DMF (9 ml) and a deoxygenated solution (saturated with N_2 over 20 min.) of **3** (505 mg, 1.17 mmol) and **4** (479 mg, 1.29 mmol) in DMF (14 ml) was added under nitrogen. The reaction mixture was stirred for 1h. The solvent was evaporated, and the residue extracted with dichloromethane (2 x 270 ml) and washed with aqueous sodium bicarbonate (5% w/v, 225 ml) and water (225 ml). Chromatography of the crude material over silica gel (1:2.5 EtOAc / hexanes, v/v) afforded the dimer **5** (711 mg, 84%), m.p. 83-86^{0}C (EtOH / H_2O): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.092 (s, 6H, $SiMe_2$), 0.90 (s, 9H, CMe_3), 1.60-1.80 (m, 2H, $^5H1^{"}$),1.92 and

1.93 (two s, 6H, 2 x 5-Me), 2.10 and 2.15 (two s, 6H, 2 x COMe), 2.18-2.26 (m, 2H, ^{3}H2'), 2.41-2.51 (m, 1H, ^{5}H3'), 2.51-2.70 (m, 2H, ^{5}H2"), 2.75 (A of ABX, 1H, $^{3}H5'_{A}$), 2.78 (B of ABX, 1H, $^{3}H5'_{B}$), 3.94 (q, 1H, ^{5}H4'), 4.10 (dt, 1H, ^{3}H4'), 4.28-4.37 (m, 3H, ^{3}H3' and $^{5}H5'_{AB}$), 5.45 (d, 1H, ^{5}H2'), 6.21 (s, 1H, ^{5}H1'), 6.21 (t, 1H, ^{3}H1'), 7.30 and 7.56 (two s, 2H, H6), 9.01 (br s, 2H, 2 x NH), $J_{3H1'-3H2'}$ = 6.6 Hz, $J_{5H2'-5H3'}$ = 6.0, $J_{5H3'-5H4'}$ = 7.5, $J_{3H3'-3H4'}$ = 4.5, $J_{3H4'-3H5'A}$ = 5.7, $J_{3H4'-3H5'B}$ = 4.9, $^{2}J_{3H5'A-3H5'B}$ = -13.8; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.11 (2 x 5-Me), 17.12 (**C**Me$_3$), 20.22 and 20.32 (2 x CO**Me**), 24.31 (^{5}C5'), 25 21 (Si**Me$_2$** and C**Me$_3$**), 30.50 (^{5}C2"), 33.49 (^{3}C5') 39.87 (^{5}C2'), 40.16 (^{3}C3'), 62.88 (^{5}C5'), 73.01 (^{3}C3'), 76.67 (^{5}C2'), 81.69, 84.54, 85.18 (^{3}H1' and 2 x H4'), 91.27 (^{5}C1'), 110.25, 110.70 (2 x C5), 135.35, 135.62 (2 x C6), 149.82, 150.10 (2 x C2), 163.79, 163.87 (2 x C4), 169.21, 169.94 (**C**OMe); MS (FAB - NBA) m/e 725 ([MH$^+$], 7.5 %), 599 ([MH$^+$ - ThyH], 52), 473 ([MH$^+$ - 2 x ThyH], 12), 399 ([MH$^+$ - 2 x ThyH - MeCOOMe], 33), 341 (57), 295 (17), 213 (100).

Diol 6

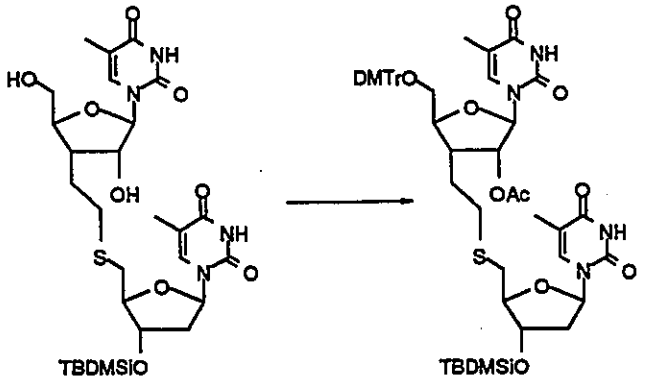


5 6

Dimer **5** (518 mg, 0.72 mmol) was suspended in dry methanol (10 ml) and cooled to 0°C. The mixture was then saturated with ammonia gas and allowed to warm to room temperature. After 11 h the resulting homogeneous solution was evaporated yielding a white foam. Chromatography over silica gel (20:1 CH_2Cl_2 / MeOH, v/v) afforded the diol **6** (442 mg, 97% yield), m.p. 108-110°C (AcOEt / hex): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.052 (s, 6H, $SiMe_2$), 0.85 (s, 9H, CMe_3), 1.55-1.70 (m, 2H, ^{5}H1"), 1.70 and 1.78 (two s, 6H, 5-Me), 2.10-2.15 (m, 6H, ^{3}H2', ^{5}H3' and ^{5}H2"), 2.95-3.12 (m, 2H, ^{3}H5'), 3.72-3.77 (m, 1H, ^{5}H4'), 3.93 (t, 1H, ^{3}H4'), 4.00-4.10 (m, 2H, ^{5}H5'), 4.44-4.46 (m, 2H, ^{3}H2' and ^{5}H2'), 5.77 (s, 1H, ^{5}H1'), 6.21 (t, 1H, ^{3}H1'), 7.51 and 7.75 (two s, 2H, H6), 9.01 (br s, 2H, 2 x NH), $J_{3H1'-3H2'}$ = 5.4 Hz, $J_{3H3'-3H4'}$ = 3.0; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.62 and 12.73 (2 x 5-Me), 18.19 (**CMe_3**), 25.73 (^{5}C1"), 26.13 (**$SiMe_2$** and **CMe_3**), 31.56 (^{5}C2"), 32.58 (^{3}C5'), 39.80 (^{5}C3'), 40.75 (^{3}C2'), 61,70 (^{5}C5'), 72.44 (^{3}C3'), 76.12(^{5}C2'), 85.59 (2 x H4'), 92.40 (2 x H1'), 109.91, 111.53 (2 x C5), 135.52, 136.62 (2 x C6), 151.19, 151.79 (2 x C2), 164.63, 164.79 (2 x C4);

MS (FAB - NBA) m/e 641 ([MH+], 18%), 515 ([MH+ - ThyH], 31), 389 ([MH+ - 2 x ThyH], 17), 257 (61), 213 (100).

Dimethoxytrityl Ether of Diol 6

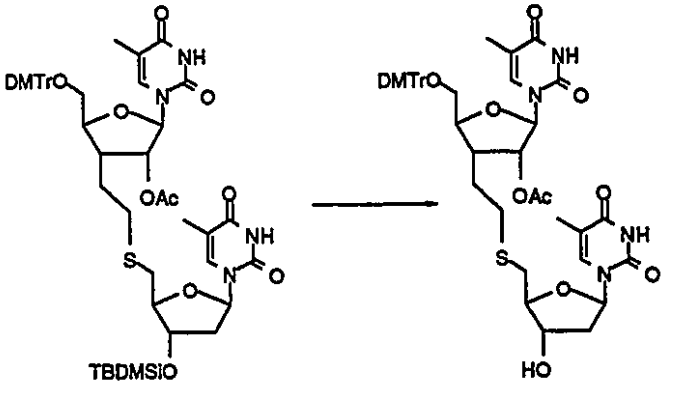


6 7

Diol **6** (220 mg, 0.34 mmole) was dissolved in dry pyridine (3.4 ml) at room temperature. Dimethoxytrityl chloride (330 mg, 0.97 mmole) and triethylamine (0.18 ml, 1.29 mmole) were added in three portions over an 8 h period. After the reaction was finished which was tested by TLC, acetic anhydride (2.00 ml, 21.2 mmole) was added and the solution was kept overnight at room temperature under nitrogen. Saturated sodium bicarbonate (30 ml) was added and the resulting solution was extracted with dichloromethane (2 x 30 ml). The combined organic layers were washed with water (30 ml), dried (Na_2SO_4) and evaporated. Chromatography over the silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) afforded the dimethoxytrityl ether **7** (0.246 mg, 76%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.078 (s, 6H, $SiMe_2$), 0.89 (s, 9H, CMe_3), 1.36-1.65 (m, 2H, ^{5}H1"),1.42 and 1.89 (two s, 6H, 5-Me), 2.14 (s, 3H, COMe), 2.08-2.26 (m, 2H,

^{3}H2'), 2.41-2.63 (m, 3H, ^{5}H3' and ^{5}H2"), 2.66-2.69 (m, 2H, ^{3}H5'), 3.21 (m, 1H, $^5H5'_A$), 3.66 (m, 1H, $^5H5'_B$), 3.78 and 3.79 (two s, 6H, 2 x COMe), 3.92 (q, 1H, ^{5}H4'), 3.99-4.02 (m, 1H, ^{3}H4'), 4.31 (dt, 1H, ^{3}H3'), 5.45 (dd, 1H, ^{5}H2'), 5.86 (d, 1H, ^{5}H1'), 6.17 (t, 1H, ^{3}H1'), 6.84 -7.43 (m, 13H, Ph), 7.30 and 7.56 (two s, 2H, 2 x H6), 9.01 (br s, 2H, 2 x NH), $J_{3H1'-3H2'}$ = 6.6 Hz, $J_{5H2'-5H3'}$ = 5.6, $J_{5H1'-5H2'}$ = 1.5, $J_{3H3'-3H4'}$ = 4.4; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 10.15 (2 x 5-Me), 16.13 (**C**Me$_3$), 20.99 (CO**Me**), 24.83 (^{5}C1"), 25.94 (Si**Me$_2$** and SiC**Me$_3$**), 31.29 (^{5}C2"), 34.13 (^{3}C5'), 39.94 (^{5}C3'), 40.50 (^{3}C2'), 46.13 (Ph$_3$**C**), 55.48 (2 x O**Me**), 62.02 (^{5}C5'), 73.52 (^{3}C3'), 77.41 (^{5}C2), 83.84, 85.13 (2 x H4'), 85.87, 89.95 (2 x H1'), 111.17, 111.44 (2 x C5), 113.51-145.68 (Ph and C6), 150.43, 150.51 (2 x C2), 164.06, 164.22 (2 x C4), 169.69 (2 x **C**OMe); MS (FAB - NBA) m/e 985 ([MH$^+$], 18%), 859 ([MH$^+$ - ThyH], 22.7), 681 ([MH$^+$ - DMTrH], 12.9), 665 ([MH$^+$ - DMTrOH], 100).

Dimethoxytritylated alcohol 8

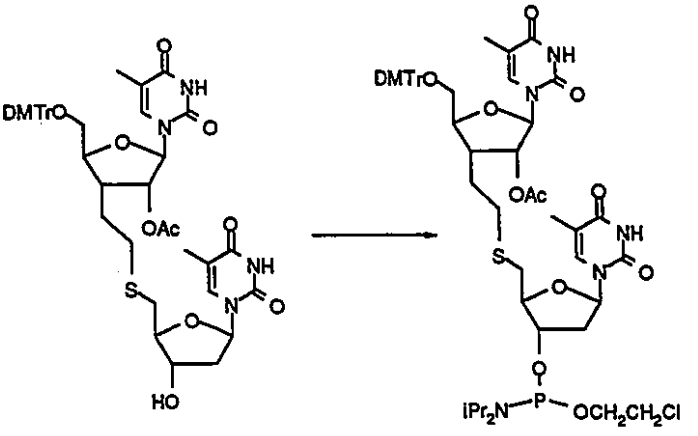


7 **8**

A solution of tetra-*n*-butylammonium fluoride in tetrahydrofuran (1 M, 0.647 mL, 0.647 mmol) was added to a stirred solution of

dimethoxytrityl ether **7** (255 mg, 0.259 mmol) and triethylamine (0.15 ml,1.17 mmole) in dry tetrahydrofuran (7 mL). After 1 h the solution was evaporated and the resulting foam was chromatographed over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) to give the dimethoxytritylated alcohol **8** (212 mg, 94%), m.p. 92-95^{0}C (EtOH / H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.38-1.62 (m, 2H, ^{5}H1"), 1.43, 1.85 (two s, 6H, 2 x 5-Me), 2.14 (s, 3H, COMe), 2.14-2.19 (m, 2H, ^{3}H2'$_A$), 2.32-2.67 (m, 6H, ^{3}H2'$_B$, ^{5}H3', ^{5}H2" and ^{3}H5'), 3.21 (m, 1H, ^{5}H5'$_A$), 3.64 (m, 1H, ^{5}H5'$_B$), 3.76 (s, 6H, 2 x COMe), 3.94-4.02 (m, 2H, ^{5}H4' and ^{3}H4'), 4.31 (dt, 1H, ^{3}H3'), 5.56 (dd, 1H, ^{5}H2'), 5.81 (d, 1H, ^{5}H1'), 6.20 (t, 1H, ^{3}H1'), 6.83 (Ph), 7.67 (s, 2H, 2 x H6), $J_{3H1'-3H2'}$ = 6.6 Hz, $J_{5H2'-5H3'}$ = 5.6, $J_{5H1'-5H2'}$ = 1.5, $J_{3H2'A-3H3'}$ = 8.0, $J_{3H2'B-3H3'}$ = 7.1, $J_{3H3'-3H4'}$ = 6.6; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 10.63 (2 x 5-Me), 21.20 (**C**OMe), 25.10 (^{5}C1"), 31.10 (^{5}C2"), 34.76 (^{3}C5'), 40.23 (^{3}C2'), 40.32 (^{5}C3'), 46.31 (Ph_3**C**), 55.61 (2 x O**M**e), 62.16 (^{5}C5'), 73.25 (^{3}C3'), 77.70 (^{5}C2), 83.91, 85.30 (2 x H4'), 90.31 (2 x H1'), 111.14 and 111.38 (2 x C5), 113.53 -144.37 (Ph and C6), 150.60, 150.74 (2 x C2), 158.88 (Ph), 164.29 , 164.49 (2 x C4), 170.05 (**C**OMe); MS (FAB - NBA) m/e 871 ([MH$^+$], 59%), 745 ([MH$^+$ - ThyH], 28), 551 ([MH$^+$ - DMTrOH], 87); HRMS (FAB - Glycerol) m/e Calcd. for $C_{45}H_{50}N_4S$ + H$^+$: 871.32242, found: 871.32203.

Phosphoramidite 9

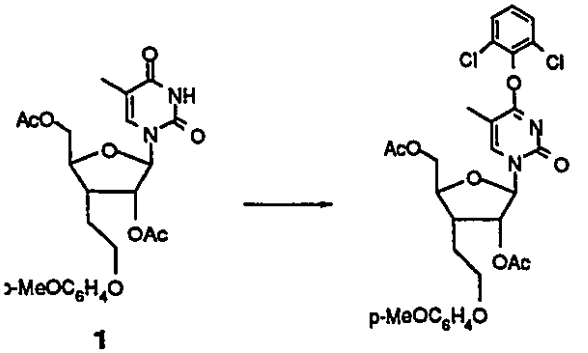


2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.137 mL, 0.62 mmol) was slowly added to a stirred solution of tritylated alcohol **8** (265 mg, 0.31 mmol) in dry dichloromethane (4 mL) containing triethylamine (0.168 mL, 1.20 mmol). After 6 h of stirring at room temperature under nitrogen, the solution was diluted with ethyl acetate (87 mL) and washed with brine (4 x 173 mL). The organic layer was then dried (Na_2SO_4), and evaporated yielding a pale yellow foam. Chromatography over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) afforded the phosphoramidite **9** as a white foam (234 mg, 72%), which was used as such in the subsequent solid-phase syntheses: ^{1}H-NMR (200 MHz, acetone) d 1.08-1.56 (m, 14H, -N(CH**Me**$_2$)$_2$ and ^{5}H1"), 1.90 and 2.05 (two s, 6H, 2 x 5-Me), 2.12 (s, 3H, OAc), 2.18-2.97 (m, 9H, ^{3}H2', ^{5}H3', ^{5}H2", ^{3}H5' and -CH_2CN), 3.23-3.65 (m, 4H, -N(**CH**$_2$Me$_2$)$_2$ and ^{5}H5'), 3.78 (s, 6H, 2 x OMe), 4.05-4.55 (m, 5H, ^{3}H4', ^{5}H4', ^{3}H3' and -POCH$_2$), 5.60 (d, 1H, ^{5}H2'), 5.80 (s, 1H, ^{5}H1'), 6.24 (t, 1H, ^{3}H1'), 6.82-7.65 (m, 15H, DMTr and 2 x H6), 10.08 (br s, 2H, 2 x NH); ^{31}P-NMR (81.0 MHz, acetone) δ 150.91 and 151.03 ppm; MS (FAB - NBA) m/e

1072 ([MH^+], 10%), 946 ([MH^+ - ThyH], 8), 853 ([MH^+ - $iPr_2NP(OH)OCH_2CH_2CN$], 5), 768 ([MH^+ - DMTrH], 9), 551 (16), 457 (100).

Experimental for Chapter 3

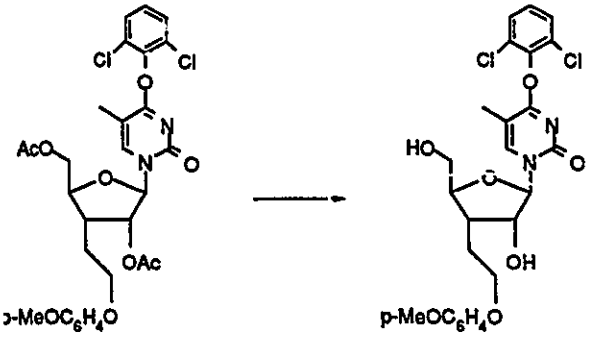
O^4-(2,6-Dichlorophenyl]-diacetate 10



To compound **1** (1.40 g, 2.94 mmole) in methylene chloride (28 ml) and triethylamine (16 ml, 114.80 mmole), was added 2-mesitylenesulfonylchloride (2.47 g, 11.29 mmole) and DMAP (192 mg, 1.56 mmole). After 30 min, 2,6-dichlorophenol (2.42 g, 14.8 mmole) and 1,4-diazabicyclo[2,2,2]octane (65 mg, 0.58 mmole) were added and stirred for another 1 hr. The solution was washed with saturated sodium bicarbonate and dried over sodium sulfate. The solvents were evaporated and the residue was purified on a silica gel column (20:1 CH_2Cl_2 /MeOH) to give **10** (1.54 g, 85%), m.p. 78-80^{0}C (EtOH / H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.78-1.88 (m, 2H, H1"), 2.15 and 2.17 (two s, 6H, OAc), 2.18 (s, 3H, 5-Me), 2.55 (h[7], 1H, H3'), 3.73 (s, 3H, OMe) 3.90 (t, 2H, H2"), 4.24 (dt, 1H, H4'), 4.48 (d, 2H, H5'), 5.58 (d, 1H, H2'), 5.81 (s, 1H, H1'), 6.75-7.40 (m, 7H, Ph) 7.88 (s, 1H, H6), $J_{H1'-H2'}$ = 1.2, $J_{H2'-H3'}$ = 5.4, $J_{H3'-H4'}$ = 10.5, $J_{H4'-H5'A}$ = 3.5, $J_{H4'-H5'B}$ = 2.5, $^2J_{H5'A-H5'B}$ = -12.8, $J_{H1"-H2"}$ = 6.0; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.39 ppm (5-Me), 20.66, 20.74 (2 x COMe), 24.41 (C1"), 38.51 (C3'), 55.58 (PhOMe), 63.12 (C5'), 65.98 (C2"), 77.40 (C2'), 82.59 (C4'), 92.34 (C1'),

103.49 (C5), 114.55-144.78 (Ph and C6), 152.39, 153.86, 154.51 (C2 and Ph), 168.68 (C4), 169.14 and 170.24 (2 x **C**OMe); MS (CI-NH_3) m/e 621 ([MH^+], 19.0 %), 351 ([MH^+ - Cl_2PhThyH], 100), 271 (40), 235 (40), 125 ([ThyH], 2.8), HRMS (CI-NH_3) m/e calcd. for $C_{29}H_{30}N_2O_9Cl_2$ + H^+: 621.14067, found: 621.14073.

O^4-[2,6,-Dichlorophenyl] diol 11

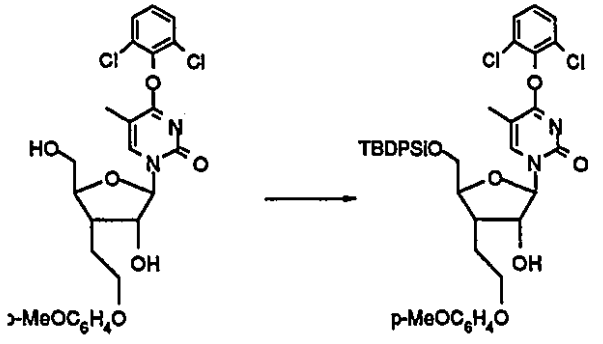


10 **11**

Diacetate **10** (1,54 g, 2.48 mmole) was dissolved in cold (0°C) methanol (17 ml) and to this solution was added a 50% aqueous methanolic solution of sodium hydroxide (1 N, 3 ml). After 1 h of stirring at 0°C, the solution was diluted with water (15 ml) and extracted with dichloromethane (3 x 20 ml). The organic layer was dried over sodium sulfate and the solvent was removed. The residue was chromatographyed (20:1 CH_2Cl_2 /MeOH) to give diol **11** (1.21 g, 91%), m.p. 196-197°C (CH_2Cl_2 / hex); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.78-1.88 (m, 2H, H1"), 2.20 (s, 3H, 5-Me), 2.33 (m, 1H, H3'), 3.73 (s, 3H, OMe), 3.98 (t, 2H, H2"), 4.05-4.18 (m, 3H, H4' and H5'), 4.25 (d, 1H, H2'), 5.69 (s, 1H, H1'), 6.75-7.40 (m, 7H, Ph), 7.88 (s, 1H, H6), $J_{H1"-H2"}$ = 6.8, $J_{H2'-H3'}$ = 4.6; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.09

ppm (5-Me), 24.19 (C1"), 36.95 (C3'), 55.86 (PhOMe), 60.16 (C5'), 67.37 (C2"), 76.62 (C2'), 86.43 (C4'), 94.05 (C1'), 104.20 (C5), 114.89-145.47 (Ph and C6), 153.16, 154.27 and 156.20 (C2 and Ph), 169.38 (C4); MS (FAB-DMSO/NBA) m/e 537 ([MH^+], 8.8 %), 271 ([Cl_2PhThyH], 100), 154 (17), 137 (38).

O^4-[2,6,-Dichlorophenyl]-5'-silyl ether 12

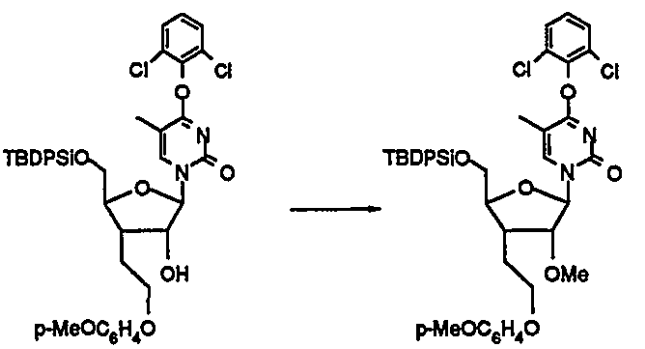


11 **12**

To a solution of diol **3** (1.21 g, 2.26 mmole) in freshly distilled DMF (4.2 ml) was added *tert*-butyldiphenylsilyl chloride (1.22 ml, 4.58 mmole) and imidazole (315 mg, 4.58 mmole). The solution was stirred for 6 h at room temperature and poured into water. The product was extracted with dichloromethane (150 ml) and washed with water (100 ml). The combined organic extracts were then dried (Na_2SO_4), filtered and the solvent removed in *vacuo*. The residue was chromatographyed over silica gel (3:1 EtOAc / hexanes v/v) to give silyl ether **12** (1.42 g, 81%), m.p. 88-90^{0}C (EtOH / H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 9H, CMe_3), 1.65 (m, 1H, H1"$_A$), 1.82 (s, 3H, 5-Me), 2.13 (m, 1H, H1"$_B$), 2.58 (h^7, 1H, H3'), 3.77 (s, 3H, OMe), 3.82-4.03 (m, 3H, H2" and H4'), 4.23 (m, 2H, H5'), 4.45 (d, 1H, H2'),

5.78 (s, 1H, H1'), 6.75-7.70 (m, 17H, Ph), 8.14 (s, 1H, H6), $J_{H2'-H3'}$ = 5.1, $J_{H3'-H4'}$ = 9.5; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.74 ppm (5-Me), 19.41 ($\mathbf{C}Me_3$), 24.07 (C1"), 27.05 ($\mathbf{C}Me_3$), 37.94 (C3'), 55.64 (O**M**e), 62.50 (C5'), 66.54 (C2"), 76.83 (C2'), 85.40 (C4'), 94.23 (C1'), 103.34 (C5), 114.58-145.05 (Ph and C6), 152.76, 153.71 and 155.51 (C2 and Ph), 169.73 (C4); MS (FAB-NBA) m/e 775 ([MH^+], 14.9 %), 717 ([MH^+ - $(CH_3)_3CH$], 22.1), 451 (14.4), 391 (10.8), 305 (14.8), 271 ([Cl_2PhThyH], 100).

O^4-[2,6,-Dichlorophenyl]-2'-O-methyl ether 13

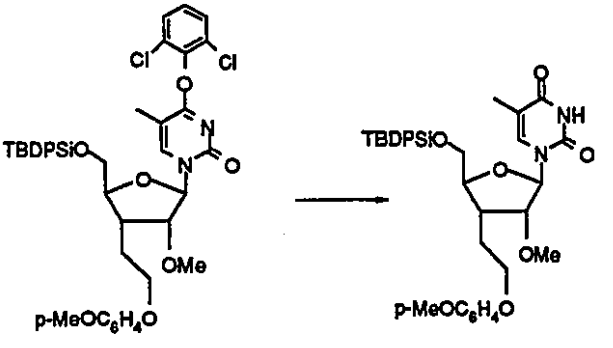


12 **13**

Sodium hydride (60% oil disp., 328 mg, 8.22 mmole) and methyl iodide (0.78 ml, 12.26 mmole) were successively added to a cooled (0^{0}C) solution of alcohol **12** (1.41 g, 1.82 mmole) in freshly distilled DMF, and the reaction mixture was stirred for 1 h at 0^{0}C. The remaining hydride was destroyed by the carefully addition of water and the resulting solution extracted with dichloromethane (3 x 60 ml). The combined organic extracts were dried (Na_2SO_4), filtered and the solvent removed in *vacuo* yielding a yellow syrup. Chromatography over silica gel (4:1 EtOAc / hexanes, v/v) afforded methyl ether **13** (1.40 g, 98%), m.p. 65-66^{0}C (EtOH /

H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 9H, CMe_3), 1.64 (m, 1H, H1"$_A$), 1.69 (s, 3H, 5-Me), 2.00 (m, 1H, H1"$_B$), 2.58 (h^7, 1H, H3'), 3.66 (s, 3H, 2'-OMe), 3.77 (s, 3H, OMe), 3.83-4.34 (m, 6H, H2", H4', H5' and H2'), 5.99 (d, 1H, H1'), 6.75-7.75 (m, 17H, Ph), 8.14 (s, 1H, H6), $J_{H1'-H2'}$ = 4.2, $J_{H2'-H3'}$ = 4.3, $J_{H3'-H4'}$ = 11.0; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.26 ppm (5-Me), 19.19 (**C**Me_3), 23.30 (C1"), 26.88 (C**Me**$_3$), 36.61 (C3'), 55.19 (PhOMe), 57.77 (2'-OMe), 61.87 (C5'), 66.04 (C2"), 84.66 (C2'), 85.03 (C4'), 89.78 (C1'), 102.73 (C5), 114.73-145.75 (Ph and C6), 152.49, 153.46, 154.53 (C2 and Ph), 168.34 (C4); MS (FAB-DMSO/NBA) m/e 789 ([MH$^+$], 14.5 %), 731 ([MH$^+$ - $(CH_3)_3CH$], 24.8), 451 (43.6), 409 ([MH$^+$ - TBDPSiOH], 23.4), 271 ([Cl_2PhThyH], 100).

2'-O-Methyl ether 14

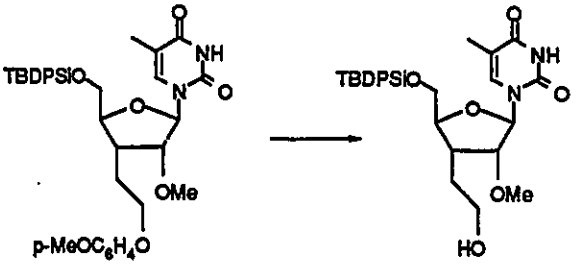


13 **14**

O^4-[2,6,-Dichlorophenyl]-2'-O-methyl ether **13** (0.84 g, 1.06 mmole) was dissolved in methanol-acetonitrile (1:1, v/v, 9 ml) and to this solution 4-nitrobenzaldoxime (1.77 g, 10.60 mmole) and 1,1,3,3-tetramethylguanidine (1.34 ml, 10.6 mmole) were added at room temperature. After 10 h, the solvent was evaporated, and the residue

dissolved in dichloromethane (30 ml) and washed with water (2 x 15 ml). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified on a silica gel column (3:1 EtOAc / hexanes, v/v) to give **14** (582 mg, 85%), m.p. 62-64^{0}C (EtOH / H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 9H, CMe_3), 1.47 (s, 3H, 5-Me), 1.68 (m, 1H, H1"$_A$), 2.01 (m, 1H, H1"$_B$), 2.60 (h^7, 1H, H3'), 3.58 (s, 3H, 2'-OMe), 3.76 (s, 3H, PhOMe), 3.78-4.23 (m, 6H, H2", H4', H5' and H2'), 5.93 (s, 1H, H1'), 6.83-8.38 (m, 14H, Ph), 8.48 (s, 1H, H6), 8.98 (s, 1H, NH), $J_{H1'-H2'}$ = 0, $J_{H2'-H3'}$ = 4.8, $J_{H3'-H4'}$ = 9.0; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.73 ppm (5-Me), 19.31 (**C**Me_3), 23.59 (C1"), 26.96 (**C**Me_3), 37.35 (C3'), 55.43 (PhOMe), 57.95 (2'-OMe), 62.11 (C5'), 66.18 (C2"), 84.93 (C2'), 85.35 (C4'), 88.65 (C1'), 110.15 (C5), 114.74-135.25 (Ph), 138.31 (C6), 150.74, 152.96 and 153.87 (C2 and Ph), 164.77(C4); MS (CI-NH_3) m/e 645 ([MH^+], 32.6%), 587 ([MH^+ - $(CH_3)_3CH$], 44.8), 307 (30.0), 127 ([ThyH + H^+], 100); HRMS (CI-NH_3) m/e calcd. for $C_{36}H_{44}N_2O_7Si$ + H^+: 645.29961, found: 645.29981.

Alcohol 15

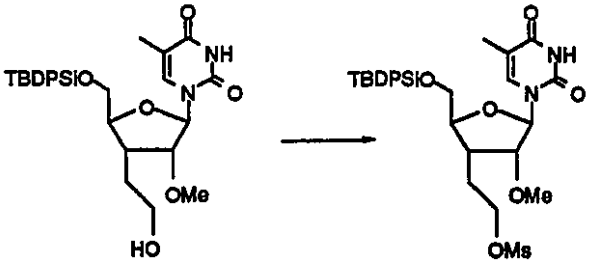


14 **15**

Ceric ammonium nitrate (1.70 g, 3.12 mmole) was added to a solution of **14** (0.52 g, 3.12 mmole) in acetonitrile-methanol (1:1.6 ml) at

0°C. The reaction was stirred at 0°C for 30 min and diluted with brine (20 ml). The mixture was extracted with ethyl acetate (3 x 40 ml). The organic extracts were washed with sodium sulfite (10% w/v, until the aqueous layer remained colorless), sodium bicarbonate (5% w/v, 30 ml), and dried (Na_2SO_4). Removal of the solvent yielded a yellow foam which was chromatographyed over silica gel (20:1 CH_2Cl_2 / MeOH, v/v), and afforded the alcohol **15** (348 mg, 72%), m.p. 68-70°C (EtOH / H_2O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 9H, CMe_3), 1.47 (s, 3H, 5-Me), 1.65 (m, 1H, H1"$_A$), 1.78 (m, 1H, H1"$_B$), 2.38 (h^7, 1H, H3'), 3.58 (s, 3H, 2'-OMe), 3.60-4.01 (m, 6H, H2", H4', H5' and H2'), 5.93 (s, 1H, H1'), 7.73-7.60 (m, 11H, Ph and H6), 10.40 (s, 1H, NH), $J_{H1'-H2'} = 0$; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.82 ppm (5-Me), 18.07 (**C**Me_3), 19.32 (C1"), 26.95 (C**Me**$_3$), 37.71 (C3'), 57.85 (2'-OMe), 60.68 (C5'), 62.60 (C2"), 85.08 (C2'), 85.44 (C4'), 88.70 (C1'), 110.00 (C5), 127.75-135.36 (C6 and Ph), 150.37 (C2), 164.70 (C4); MS (CI-NH_3) m/e 539 ([MH^+], 48.7%), 481 ([MH^+ - $(CH_3)_3CH$], 31.2), 355 ([MH^+ - ThyH], 8.6), 241 (100), 127 ([ThyH + H^+], 100); HRMS (CI-NH_3) m/e calcd. for $C_{29}H_{38}N_2O_6Si$ + H^+: 539.25775, found: 539.26490.

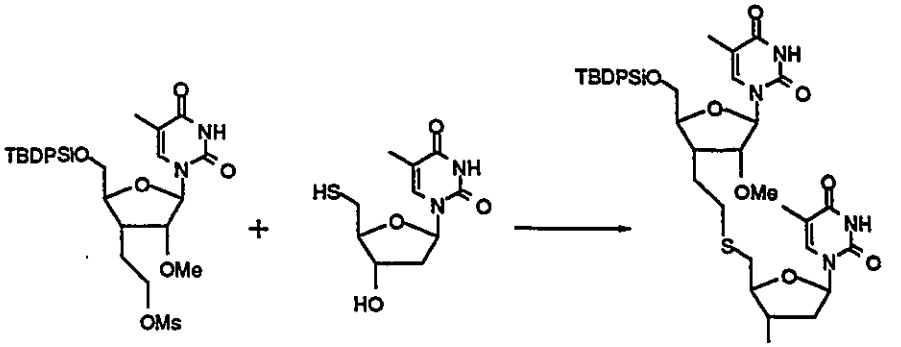
Mesylate 16



15 **16**

Methanesulfonyl chloride (0.14 ml, 1.83 mmole) was added to a stirred solution of alcohol **15** (0.43 g, 0.80 mmole) and dry triethylamine (0.30 ml, 2.15 mmole) in dry dichloromethane (7 ml) at room temperature under nitrogen. After 1 h, the reaction was diluted with dichloromethane (10 ml) and washed with hydrochloric acid (5% w/v, 2 ml), saturated aqueous sodium bicarbonate (2 ml), and brine (5% w/v, 2 ml). The organic layer was dried (Na_2SO_4), and the solvent was removed yielding a yellow foam. Chromatography over silica gel (20 : 1 CH_2Cl_2 / MeOH, v/v) afforded the mesylate **16** as a white foam (406 mg, 87%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.07 (s, 9H, CMe_3), 1.53 (s, 3H, 5-Me), 1.60 (m, 1H, H1"$_A$), 1.98 (m, 1H, H1"$_B$), 2.50 (h[7], 1H, H3'), 2.96 (s, 3H, SO_2CH_3), 3.57 (s, 3H, 2'-OMe), 3.77 (dd, 1H, H2"$_A$), 3.88 (d, 1H, H4'), 4.02 (d, 1H, H2"$_B$), 4.17-4.28 (m, 3H, H5' and H2'), 5.94 (s, 1H, H1'), 7.37-7.82 (m, 11H, Ph and H6), 9.34 (br s, 1H, NH), $J_{H2'-H3'}$ = 4.7, $J_{H3'-H4'}$ = 9.6, $J_{H1"-H2"A}$ = 12, $J_{H1"-H2"B}$ = 10.5, $J_{H2"A-H2"B}$ = 2.2; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.01 ppm (5-Me), 19.37 (**C**Me_3), 23.84 (C1"), 26.99 (**C**Me_3), 37.26 (C3'), 58.00 (2'-OMe), 60.24 (C5'), 62.15 (C2"), 67.70 (SO_2CH_3), 84.46 (C2'), 84.91 (C4'), 88.59 (C1'), 110.52 (C5), 127.72-135.33 (C6 and Ph), 150.33 (C2), 164.14 (C4); MS (FAB - NBA) m/e 539 ([MH^+], 5.8 %), 559 ([MH^+ - $(CH_3)_3CH$], 13.0), 307 (11.9), 135 (100); HRMS (FAB - glycerol) m/e calcd. for $C_{30}H_{40}N_2O_8SSi$ + H^+: 617.23530, found: 617.23527.

Dimer 18

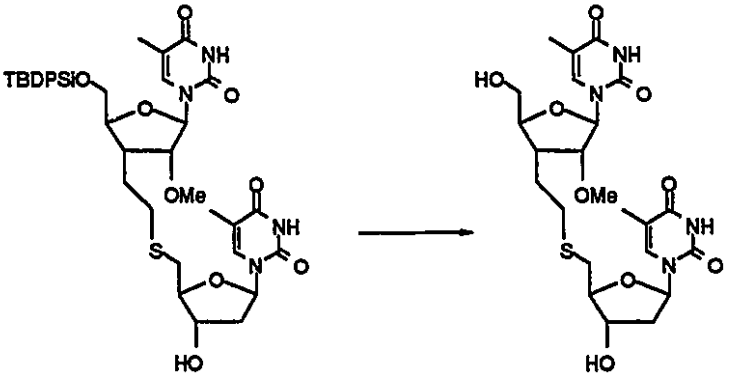


16 17 18

Cesium carbonate (76 mg, 0.43 mmol) previously flame dried *in vacuo*, was suspended in dry DMF (1.5 ml) and a deoxygenated solution (saturated with N_2 over 20 min.) of mesylate **16** (97 mg, 0.16 mmol) and thiol **17** (61 mg, 0.24 mmol) in dry DMF (2 ml) was then added, resulting in a yellow solution. After 1 h of stirring at room temperature under nitrogen, the solvent was evaporated and the product was dissolved in dichloromethane (2 x 15 ml) and washed with aqueous sodium bicarbonate (5% w/v, 5 ml) and water (5 ml). The combined organic layers were dried (Na_2SO_4) and evaporated. Chromatography of the crude product over silica gel (1:2.5 EtOAc / hexanes, v/v) afforded the dimer **18** (86 mg, 84%), m.p. 116-118^{0}C (AcOEt / hex): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.07 (s, 9H, CMe_3), 1.44 (s, 3H, ^{3}5-Me), 1.74-1.87 (m, 2H, ^{5}H1"), 1.88 (s, 3H, ^{5}5-Me), 2.18-2.41 (m, 3H, ^{3}H2' and ^{5}H3'), 2.44-2.62 (m, 2H, ^{5}H2"), 2.79 (d, 2H, ^{3}H5'), 3.55 (s, 3H, ^{5}2'-OMe), 3.73 (dd, 1H, ^{5}H4'), 3.85 (d, 1H, ^{3}H4'), 3.97-4.02 (m, 2H, $^5H5'_A$ and ^{5}H2'), 4.18 (d, 1H, $^5H5'_B$), 4.35 (dt, 1H, ^{3}H3'), 5.85 (s, 1H, ^{5}H1'), 6.21 (t, 1H, ^{3}H1'), 7.25-7.63 (m, 12H, Ph and 2 x H6), 9.23 and 9.43 (br s, 1H, 2 x NH), $J_{5H1'-5H2'}$ = 0, $J_{5H2'-5H3'}$ = 5.0, $J_{5H3'-5H4'}$ = 2.5, $J_{3H1'-3H2'}$ = 6.6, $J_{3H3'-}$

$J_{3H4'}$ = 5.0, $J_{3H2''-3H3'}$ = 7.2; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.94 and 12.54 ppm (2 x 5-Me), 19.41 (CMe_3), 23.95 (^{5}C1"), 27.03 (CMe_3), 31.38 (^{5}C2"), 34.42 (^{3}C5'), 39.80 (^{5}C3'), 39.93 (^{3}C2'), 58.02 (^{5}2'-OMe), 62.49 (^{5}C5'), 73.07 (^{3}C3'), 84.61, 84.68, 85.06 (^{5}C2', ^{3}C1' and 2 x C4'), 88.66 (^{5}C1'), 110.32, 111.05 (2 x C5), 127.72-135.61 (C6 and Ph), 150.49 (2 x C2), 164.01, 164.34 (2 x C4); MS (FAB - NBA) m/e 779 ([MH^+], 5.1 %), 653([MH^+ - ThyH], 23.0), 509 ([MH^+ - $TBDPSiOCH_3$], 6.7), 307 (21), 135 (100), 127 ([ThyH + H^+], 37.9); HRMS (FAB-glycerol) m/e calcd. for $C_{39}H_{50}N_4O_9SSi$ + H^+: 779.31461, found: 779.31438

Diol 19

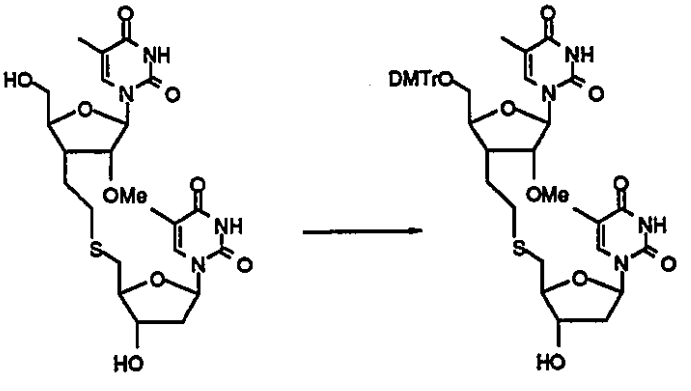


18 **19**

A solution of tetra-butylammonium fluoride in THF (1M, 0.20 ml, 0.20 mmole) was added to a solution of dimer **18** (80 mg, 0.10 mmole) in dry THF (0.7 ml). After 3 h the solution was evaporated and the resulting syrup was chromatographyed over silica gel (20:1 CH_2Cl_2 / MeOH, v/v) giving diol **19** (45 mg, 81%), m.p. 167-169^{0}C (CH_2Cl_2 / hex): ^{1}H-NMR (200 MHz, $CDCl_3$ + CD_3OD) d 1.52-1.87 (m, 2H, ^{5}H1"), 1.88 and 1.91 (2s, 6H, 2 x 5-

Me), 2.05-2.16 (m, 3H, ^{3}H2' and ^{5}H3'), 2.51 (m, 2H, ^{5}H2"), 2.80 (d, 2H, ^{3}H5'), 3.55 (s, 3H, ^{5}2'-OMe), 3.73 (m, 1H, ^{5}H4'), 3.95-4.08 (m, 4H, ^{3}H4', ^{5}H5' and ^{5}H2'), 4.21 (dt, 1H, ^{3}H3'), 5.80 (s, 1H, ^{5}H1'), 6.08 (t, 1H, ^{3}H1'), 7.28, 8.05 (two s, 2H, 2 x H6), $J_{5H1'-5H2'} = 0$, $J_{3H1'-3H2'} = 6.6$, $J_{3H3'-3H4'} = 4.8$; ^{13}C-NMR ($CDCl_3$ + CD_3OD) δ 12.02 and 12.24 ppm (2 x 5-Me), 23.82 (^{5}C1"), 30.65 (^{5}C2"), 34.08 (^{3}C5'), 38.43 (^{5}C3'), 39.49 (^{3}C2'), 57.70 (^{5}2'-OMe), 59.47 (^{5}C5'), 72.30 (^{3}C3'), 84.67, 85.02 and 85.38 (^{5}C2', ^{3}C1' and 2 x C4'), 88.16 (^{5}C1'), 109.55 and 110.96 (2 x C5), 135.76 and 136.49 (2 x C6), 150.34 and 150.45 (2 x C2), 164.33 and 164.45 (2 x C4); MS (FAB - NBA) m/e 541 ([MH$^+$], 23.6 %), 415 ([MH$^+$ - ThyH], 15.4), 289 ([MH$^+$ - 2 x ThyH], 50.4), 246 (100); HRMS (FAB-glycerol) m/e calcd. for $C_{23}H_{32}N_4O_9S$ + H$^+$: 541.19683, found: 541.19668.

Dimethoxyltrityl ether 20

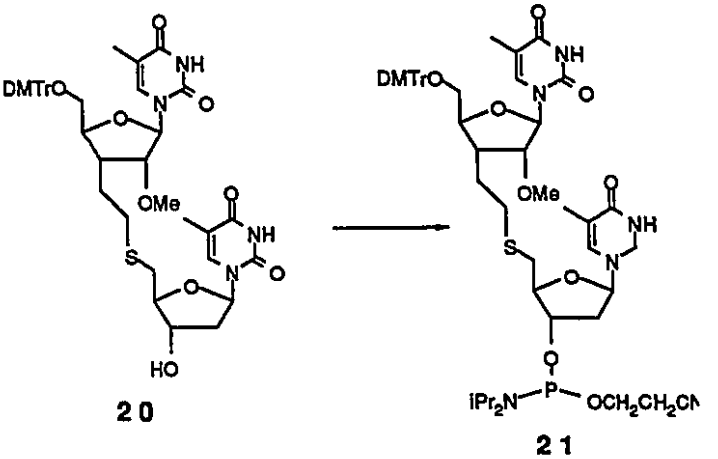


19 **20**

4,4-Dimethoxytrityl chloride (163 mg, 0.48 mmole) was added to a solution of diol **19** (130 mg, 0.24) in dry pyridine (3.5 ml) and triethylamine (0.17 ml, 1.24 mmole) at room temperature. After 14 h,

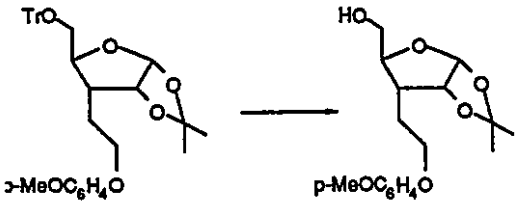
saturated sodium bicarbonate (20 ml) was added and the resulting solution was extracted with dichloromethane (2 x 20 ml). The combined organic layers were washed with water (20 ml), dried (Na_2SO_4) and evaporated. The resulting syrup was chromatographyed over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) giving **20** (168 mg, 83%), m.p. 78-81^{0}C (EtOH /H_2O): ^{1}H-NMR (200 MHz, acetone) δ 1.54 (s, 3H, ^{3}5-Me), 1.45-1.52 (m, 2H, ^{5}H1"), 1.80 (s, 3H, ^{5}5-Me), 2.05 (t, 2H, ^{3}H2') 2.21-2.78 (m, 5H, ^{5}H3', ^{5}H2" and ^{3}H5'), 3.26 (m, 1H, ^{5}H4'), 3.55 (s, 3H, ^{5}2'-OMe), 3.65 (m, 1H, ^{3}H4'), 3.78 (s, 6H, 2 x PhOMe), 3.96-4.05 (m, 3H, ^{5}H5' and ^{5}H2'), 4.38 (dt, 1H, ^{3}H3'), 5.88 (s, 1H, ^{5}H1'), 6.29 (t, 1H, ^{3}H1'), 6.94-7.81 (m, 11H, Ph and 2 x H6), $J_{5H1'-5H2'}$ = 0, $J_{3H1'-3H2'AB}$ = 6.8 and 7.0, $J_{3H2'AB-3H3'}$ = 1.9, $J_{3H3'-3H4'}$ = 3.6; ^{13}C-NMR (75.4 MHz, acetone) δ 11.78, 11.92 ppm (2 x 5-Me), 26.18 (^{5}C1"), 30.69 (^{5}C2"), 34.84 (^{3}C5'), 39.81 (^{5}C3'), 40.97 (^{3}C2'), 46.34 (Ph_3**C**), 55.49 (PhOMe), 58.45 (^{5}2'-OMe), 62.60 (^{5}C5'), 73.91 (^{3}C3'), 84.65, 85.15, 86.31 (^{5}C2', ^{3}C1' and 2 x C4'), 87.15 (^{5}C1'), 109.99, 110.90 (2 x C5), 127.20-145.56 (Ph and 2 x C6), 151.05, 151.21 (2 x C2), 159.67, 164.36 (2 x C4); MS (FAB - NBA) m/e 944 ([M + Et_3NH^+], 4.7 %), 842 ([M], 2.0), 460 (10.1), 391 (16.5), 303 ([DMTrH], 100); HRMS (FAB-glycerol) m/e calcd. for $C_{44}H_{50}N_4O_{11}S$ + H^+: 843.32751, found: 843.32776.

Amidite 21



2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.085 mL, 0.38 mmol) was slowly added to a stirred solution of tritylated alcohol **20** (160 mg, 0.19 mmol) in dry dichloromethane (1 mL) containing triethylamine (0.065 mL, 0.46 mmol). After 12 h of stirring at room temperature under nitrogen, the solvent was evaporated yielding a pale yellow foam. The crude product was chromatographyed over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) affording the phosphoramidite **21** as a colorless foam (195 mg, 95%), which was used as such in the subsequent solid-phase syntheses: ^{1}H-NMR (200 MHz, acetone) d 1.08-1.56 (m, 14H, -N(CH**Me**$_2$)$_2$ and ^{5}H1"), 1.80 (s, 6H, 2 x 5-Me), 2.45 (m, 1H, 5H3'), 2.48-2.85 (m, 8H, ^{3}H2', ^{5}H2", ^{3}H5' and -CH_2CN), 3.26 (m, 1H, 5H2'), 3.52 (s, 6H, 2 x 2-OMe), 3.78 (s, 6H, 2 x PhOMe), 3.60-4.20 (m, 8H, ^{5}H5',^{3}H4', ^{5}H4', -N(**CH**$_2$Me$_2$)$_2$ and -POCH$_2$), 4.58 (m, 1H, ^{3}H3'), 5.83 (s, 1H, ^{5}H1'), 6.22 (t, 1H, ^{3}H1'), 6.82-7.81 (m, 15H, DMTr and 2 x H6), 10.08 (br s, 2H, 2 x NH); ^{31}P-NMR (81.0 MHz, acetone) δ 150.85 and 151.03 ppm; MS (FAB - NBA) m/e 1043 ([MH$^+$], 0.5%), 399 (69.7), 303 ([DMTr$^+$], 100), 219 ([*i*Pr$_2$NH$^+$P(OH)OCH$_2$CH$_2$CN], 25.2).

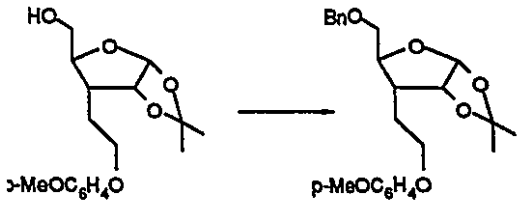
Alcohol 23



22 **23**

Trityl ether **22** (668 mg, 1.18 mmole) was dissolved in dichloromethane (16 ml). To this solution was added dropwise a solution of trichloroacetic acid in dichloromethane (1:4 w/v, 5.6 ml). After 3 h of stirring at room temperature, the reaction mixture was diluted with chloroform (30 ml), washed with saturated aqueous sodium bicarbonate (60 ml) and water (60 ml). The organic phase was then dried (Na_2SO_4), filtered and evaporated. Chromatography of the crude product over silica gel (1:1 EtOAc / hexanes, v/v) afforded alcohol **23** (271 mg, 71%) as a colorless syrup: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.34-1.52 (two s, 6H, CMe_2), 1.80-1.92 (m, 2H, H1'), 2.05 (m, 1H, -OH), 2.28 (h^7, 1H, H3), 3.60 (A of ABX, 1H, $H5_A$), 3.78 (s, 3H, OMe), 3.88-4.08 (m, 4H, H4, $H5_B$ and H2'), 4.70 (t, 1H, H2), 5.82 (d, 1H, H1), 6.84 (s, 4H, Ph), J_{H1-H2} = 2.6, J_{H2-H3} = 5.3, J_{H3-H4} = 6.7, J_{H4-H5A} = 2.8, $^2J_{H5'A-H5'B}$ = -10.5; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 24.73 (C1'), 26.35, 26.71 (CMe_2), 40.71 (C3), 55.66 (OMe), 61.68 (C5), 66.54 (C2'), 81.34 (C2), 82.14 (C4), 104.92 (C1), 111.66 (CMe_2), 114.60-153.82 (CPh); MS (CI-NH_3) m/e 342 ([M + NH_4^+], 5.3%), 324 ([M], 15.2), 267 ([MH^+ - C_3H_6O], 87.7), 124 ([$C_7H_7O_2$], 100), HRMS (CI-NH_3) m/e calcd. for $C_{17}H_{24}O_6$ + NH_4^+: 343.19949, found: 343.19020.

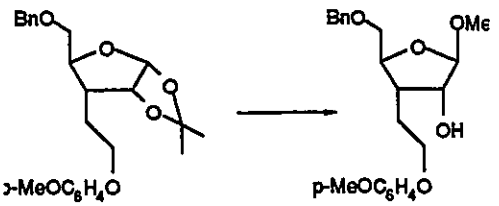
Benzyl Ether 24



23 **24**

Sodium hydride (60% oil disp., 35 mg, 0.81 mmole) was added to a solution of alcohol **23** (260 mg, 0.80 mmole) in THF (1.5 ml) at room temperature, and the reaction mixture was stirred for 30 min. Then tetra-butylammonium iodide (30 mg, 0.08 mmole) and benzyl bromide (0.19 ml, 1.60 mmole) were added and the mixture was stirred for another 5 h. The reaction was quenched with water (5 ml) and extracted with dichloromethane (2 x 15 ml). The combined organic phases were washed with brine (5 ml), dried (Na_2SO_4), filtered and evaporated. The resulting syrup **24** was used as such in next step. An analytical sample was obtained by chromatography over silica gel (2:1 EtOAc / hexanes, v/v): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.32, 1.51 (two s, 6H, CMe_2), 1.75-2.08 (m, 2H, H1'), 2.29 (h^7, 1H, H3), 3.52 (A of ABX, 1H, $H5_A$), 3.68-3.76 (overlapping s and m, 4H, OMe and $H5_B$), 3.93-4.12 (m, 3H, H4 and H2'), 4.58 (d, 2H, $PhCH_2$, J=7.0 Hz), 4.66 (t, 1H, H2), 5.84 (d, 1H, H1), 6.82-7.32 (m, 9H, Ph); J_{H1-H2} = 3.8, J_{H2-H3} = 4.4, J_{H4-H5A} = 4.3, $^2J_{H5'A-H5'B}$ = -10.8; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 24.80 (C1'), 26.40, 26.73 (CMe_2), 41.67 (C3), 55.71 (OMe), 66.44 (C5), 69.31 (C2'), 73.42 ($PhCH_2$), 80.90 (C2 and C4), 105.00 (C1), 111.48 (CMe_2), 114.60-153.82 (CPh); MS (CI-NH_3) m/e 415 ([MH^+], 6.8%), 414 ([M], 24.3), 375 ([$MH^+ - C_3H_6O$], 49.7), 215 (62), 142 (100).

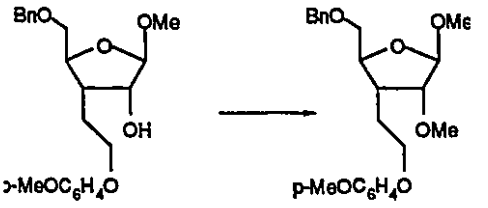
Alcohol 25



24 **25**

Camphorsulfonic acid (562 mg, 2.42 mmole) was added to a stirred methanolic solution (10 ml) of benzyl ether **24** prepared above (~0.8 mmol) The solution was refluxed for 15 min, cooled in ice and slowly added to aqueous sodium bicarbonate (5%, 30 ml). The mixture was stirred over 30 min and extracted with dichloromethane (2 x 100 ml). The combined organic layers were then dried (Na_2SO_4), filtered and evaporated, yielding a yellow syrup **25** which was ready to be used for next step. An analytical sample was obtained by chromatography over silica gel (1:4 EtOAc / hex, v/v): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.82-2.13 (m, 2H, H1'), 2.25 (m, 1H, H3), 3.33 (s, 3H, 1-OMe), 3.52 (d, 2H, H5), 3.77 (s, 3H, OMe), 3.82-4.20 (m, 4H, H2, H4 and H2'), 4.58 (s, 2H, $PhCH_2$), 4.90 (s, 1H, H1), 6.91-7.48 (m, 9H, Ph), J_{H1-H2} = 0; ^{13}C-NMR (55.5 MHZ, $CDCl_3$) δ 25.53 (C1'), 42.66 (C3), 54.17 (1-OMe), 55.42 (Ph**OMe**), 67.67 (C5), 73.05 (C2'), 73.35 (Ph**CH_2**), 82.61 (C2), 86.81 (C4), 108.90 (C1), 114.45-156.03 (CPh); MS (CI-NH_3) m/e 406 ([M + NH_4^+], 7.3%), 388 ([M], 28.5), 357 ([MH^+ - C_3H_6O], 69.8), 124 ([C_7H_8O], 100).

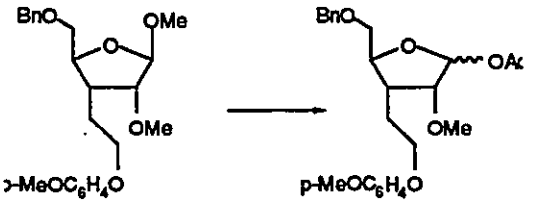
2-O-methyl ether 26



25 **26**

To a cooled (0^0C) solution of alcohol **25** prepared above (~0.8 mmol) in DMF (3.3 ml) was added sodium hydride (60% oil disp., 40 mg, 1.01 mmole), and the reaction mixture was stirrd for 45 min at 0^0C. Then methyl iodide (0.06 ml, 0.93 mmole) was added, and stirring continued for another 45 min at 0^0C. The excess sodium hydride was destroyed by slowly adding water, and the mixture extracted with ethyl acetate (2 x 40 ml). The combined organic layers were dried (Na_2SO_4), filtered and evaporated. Chromatography of the crude product over silica gel (1:4 AcOEt / hexanes, v/v) gave colorless syrup **26** (212 mg, 66% yield from **23**, via **24** and **25**): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.77-2.22 (m, 2H, H1'), 2.35 (m, 1H, H3), 3.38 (s, 3H, 1-OMe), 3.42 (s, 3H, 2-OMe), 3.47-3.62 (overlapping A and B of ABX, 2H, H5), 3.64 (d, 1H, H2), 3.75 (s, 3H, PhOMe), 3.88 (t, 2H, H2'), 4.07-4.14 (m, 1H, H4), 4.61 (s, 2H, $PhCH_2$), 4.95 (s, 1H, H1), 6.83-7.40 (m, 9H, Ph); J_{H1-H2} = 0, J_{H2-H3} = 3.2, J_{H4-H5A} = 4.2, J_{H4-H5B} = 2.6, $^2J_{H5'A-H5'B}$ = -9.6; ^{13}C-NMR (55.5 MHZ, $CDCl_3$) δ 25.41 (C1'), 40.51 (C3), 54.45 (1-OMe), 55.72 (PhOMe), 57.94 (2-OMe), 67.17 (C5), 73.25 (C2'), 73.51 (Ph**CH_2**), 83.15 (C2), 85.06 (C4), 1005.46(C1), 114.63-153.76 (CPh); MS (CI-NH_3) m/e 420 ([M + NH_4^+], 6.8%), 402 ([M], 25.9), 371 ([MH^+ - C_3H_6O], 100), 157 (100).

Acetate 27

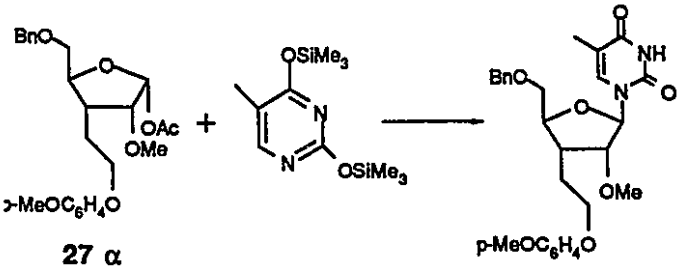


26 **27**

To a stirred solution of **26** (148 mg, 0.37 mmole) in 1,2-dichloroethane (4 ml) was added dimethylboron bromide (0.60 ml, 1.20 mmole) at -78^{0}C under nitrogen. After 1.5 h, acetic acid (0.30 ml, 5.23 mmole) and triethylamine (1.00 ml, 6.75 mmole) were added to the reaction mixture, then the solution was warmed to 0^{0}C and stirred for another 30 min. The reaction solution was diluted with CH_2Cl_2 (5 ml) and washed with saturated $NaHCO_3$. The aqueous solution was extracted with CH_2Cl_2 (2 x 5 ml). The combined organic layers were dried (Na_2SO_4), filtered and evaporated. The crude compound was chromatographyed over silica gel (1:3 hex / AcOEt) to give **27** (140 mg, 88%, α : β = 6:1 by NMR) as a colorless syrup. The α-isomer was obtained by once more carefully chromatography (1:1 hex / AcOEt). For α-isomer: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.85 (m, 1H, H1'$_A$), 2.09 (d, 3H, OAc, J=1.0 Hz), 2.17 (m, 1H, H1'$_B$), 2.48 (m, 1H, H3), 3.38 (d, 3H, 2-OMe, J=1.1 Hz), 3.46-3.61 (overlapping A and B of ABX, 2H, H5), 3.75 (d, 3H, PhOMe, J=0.9 Hz), 3.89-3.97 (m, 3H, H2' and H2), 4.30 (q, 1H, H4), 4.52 (d, 2H, $PhCH_2$, J=2.1 Hz), 6.36 (d, 1H, H1), 6.77-7.28 (m, 9H, Ph), J_{H1-H2} = 4.0, J_{H2-H3} = 4.5, J_{H4-H5A} = 4.4, J_{H4-H5B} = 4.6, $^2J_{H5'A-H5'B}$ = -10.5; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 21.38 (CO**Me**), 27.76 (C1'), 37.68 (C3), 55.71 (PhO**Me**), 59.15 (2-OMe), 67.38 (C5), 71.40 (C2'), 73.46 (Ph**CH$_2$**), 83.06 (C2), 84.23 (C4), 95.61 C1), 114.61-153.75 (CPh), 170.25 (**C**OMe); MS

(CI-NH_3) m/e 438 ([M + NH_4^+], 7.4%), 430 ([M], 11.5), 371 ([M - CH_3COO^-], 100), 157 (100); HRMS (FAB-Glycerol) m/e calcd. for $C_{24}H_{30}O_7$ + H^+: 431.20698, Found 431.20709.

2-O-methyl thymidine analogue 28



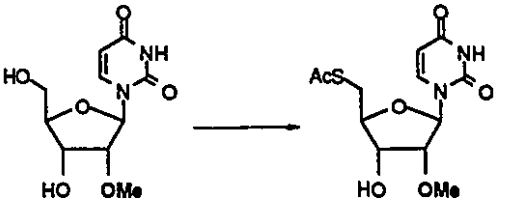
27 α **28**

To a cooled (0^0C) solution of acetate **27α** (116 mg, 0.27 mmole) and disilylated thymine (77 mg, 0.28 mmole) in 1,2-dichloroethane (1.6 ml), a solution of redistilled $SnCl_4$ (0.02ml, 0.19 mmole) in 1,2-dichloroethane was added with vigorous stirring. The reaction was finished after 16 h at room temperatures, and the solution was diluted with dichloromethane (5 ml) and washed with saturated $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4) and evaporated. The crude product was purified over a silica gel column (1:3 hex / AcOEt) to give **28** (64 mg, 48%) as a white foam: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.51 (s, 3H, 5-Me), 1.70 (m, 1H, H1"$_A$), 2.05 (m, 1H, H1"$_B$), 2.52 (h[7], 1H, H3'), 3.58 (s, 3H, 2'-OMe), 3.64 (m, 2H, H5'), 3.76 (s, 3H, PhOMe), 3.90-4.26 (m, 4H, H2", H2' and H4'), 4.46 (d, 2H, $PhCH_2$, J = 2.1 Hz), 5.91 (s, 1H, H1'), 6.78-7.35 (m, 9H, Ph), 7.88 (s, 1H, H6), 8.30 (s, 1H, NH); J_{H1-H2} = 0, J_{H2-H3} = 4.0; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 11.71 ppm (5-Me), 23.60 (C1"), 36.78 (C3'), 55.03 (PhOMe), 57.28 (2'-OMe), 65.82

(C5'), 66.80 (C2"), 72.64 (Ph$\mathbf{CH_2}$), 83.26 (C2'), 84.75 (C4'), 87.31 (C1'), 109.70 (C5), 113.11-127.04 (Ph), 134.41 (C6) 150.90 (C2), 161.73 (C4); MS (CI-NH_3) m/e 497 ([MH^+], 34.9%), 371 ([MH^+ - ThyH], 13.0), 127 ([ThyH + H^+], 100), HRMS (FAB-Glycerol) m/e calcd. for $C_{27}H_{32}N_2O_7$ + H^+: 497.22878, found: 497.22900.

Experimental for Chapter 4

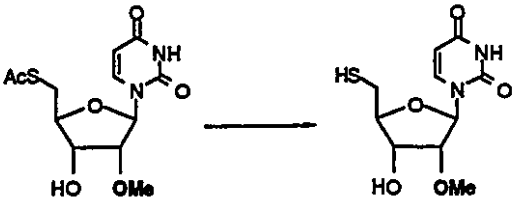
Thioester 30



29 30

Diisopropyl azodicarboxylate (1.53 ml, 7.80 mmole) was added dropwise to a cooled (0°C) solution of triphenylphosphine (2.05 g, 7.84 mmole) in dry THF (29 ml) resulting in a milky suspension. After 0.5 h, a solution of diol **29** (1.00 g, 3.88 mmole) and thioacetic acid (0.56 ml, 7.84 mmole) in THF was slowly added to the reaction mixture above. The reaction was stirred at 0°C for 3 h and the solvent removed in *vacuo*. Chromatography over silica gel (10:1 CH_2Cl_2 / MeOH, v/v) afforded thioester **30** (832 mg, 82%); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.39 (s, 3H, COMe), 3.25 (A of ABX, 1H, $H5'_A$), 3.32 (B of ABX, 1H, $H5'_B$), 3.55 (s, 3H, 2'-OMe), 3.76-4.06 (m, 3H, H2', H3' and H4'), 5.80 (overlapping s and d, 2H, H5 and H1'), 7.45 (d, 1H, H6'), 10.05 (broad s, 1H, NH), $J_{H4'-H5'A}$ = 6.6, $J_{H4'-H5'B}$ = 4.2, $^2J_{H5'A-H5'B}$ = -12, J_{H5-H6} = 8.2; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 30.59 ppm (COMe and C5'), 58.64 (2'-OMe), 71.78 (C3'), 81.83 (C2'), 83.08 (C4'), 88.39 (C1'), 102.65 (C5), 138.79 (C6), 150.11 (C2), 183.63 (C4), 195.84 (COMe); MS (CI-NH_3) m/e 317 ([MH^+], 45.7 %), 247 (22.1), 241 ([MH^+ - CH_3COSH], 19.9), 113 ([UraH + H^+], 100).

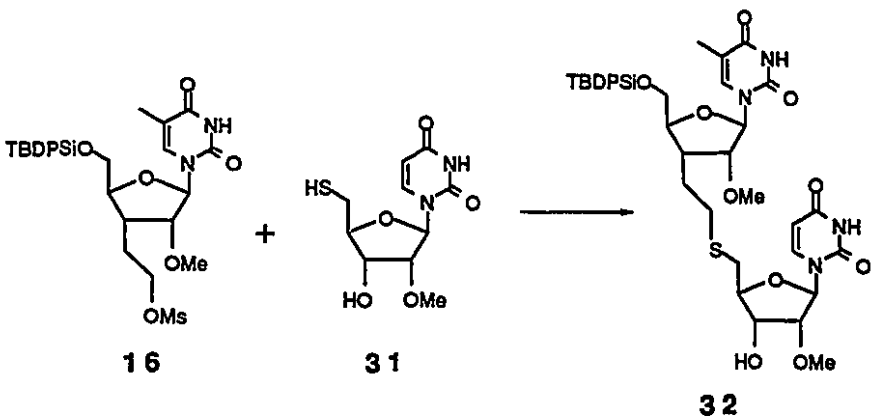
Thiol 31



30 **31**

Thioester **30** (518 mg, 1.64 mmole) was dissolved in methanol (15 ml) cooled in an ice-bath and NH_3 gas was bubbled into the solution under iced-bath for 10 min. The solution was stirred for 3 h at room temperature and the solvent was removed. The residue was purified on a silica gel column (20:1 CH_2Cl_2/MeOH, v/v) and yielded thiol **31** (391 mg, 87%); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.80 (t, 1H, SH), 2.86 (A of ABXY, 1H, H5'$_A$), 3.00 (B of ABXY, 1H, H5'$_B$), 3.58 (s, 3H, 2'-OMe), 3.81 (dd, 1H, H2'), 4.01 (dt, 1H, H4'), 4.10 (m, 1H, H3'), 5.77 (d, 1H, H5), 5.88 (d, 1H, H1'), 7.63 (d, 1H, H6'), 10.20 (broad s, 1H, NH), $J_{H1'-H2'}$ = 2.1, $J_{H2'-H3'}$ = 5.6, $J_{H3'-H4'}$ = 7.4, $J_{H4'-H5'A}$ = 4.5, $J_{H4'-H5'B}$ = 3.9, $^2J_{H5'A-H5'B}$ = -13.2, $J_{SH-H5'A}$ = 8.5, $J_{SH-H5'B}$ = 8.8, J_{H5-H6} = 8.1; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 25.88 ppm (C5'), 58.73 (2'-OMe), 70.38 (C3'), 82.37 (C2'), 83.07 (C4'), 87.87 (C1'), 102.52 (C5), 139.99 (C6), 150.12 (C2), 163.68 (C4); MS (CI-NH_3) m/e 275 ([MH^+], 60.3%), 113 ([UraH = H^+], 100), HRMS (CI-NH_3) m/e calcd. for $C_{10}H_{14}N_2O_5S$ + H^+: 275.07018, found: 275.07005.

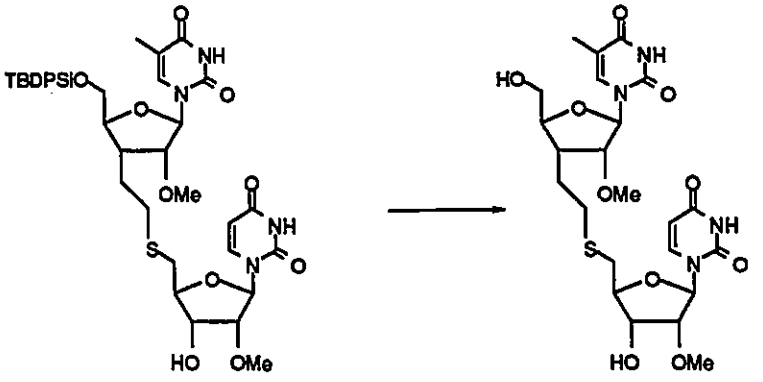
Dimer 32



Cesium carbonate (70 mg, 0.42 mmol), previously flame dried in vacuo, was suspended in dry DMF (1.3 ml) and a deoxygenated solution (saturated with N_2 over 20 min.) of mesylate **16** (89 mg, 0.15 mmol) and thiol **31** (60 mg, 0.22 mmol) in dry DMF (2 ml) was then added, resulting in a yellow solution. After 1 h of stirring at room temperature under nitrogen, the solvent was removed and the product was dissolved in dichloromethane (2 x 15 ml) and washed with aqueous sodium bicarbonate (5% w/v, 5 ml) and water (5 ml). The combined organic layers were dried (Na_2SO_4) and evaporated, yielding a yellow foam. Chromatography over silica gel (1:2.5 ethyl acetate / hexanes, v/v) afforded the dimer **32** (94 mg, 80%), m.p. 97-98°C (EtOH / H_2O): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.07 (s, 9H, CMe_3), 1.44 (s, 3H, ^{5}5-Me), 1.70-1.91 (m, 2H, ^{5}H1"), 2.31 (m, 1H, ^{5}H3'), 2.47-2.63 (m, 2H, ^{5}H2"), 2.75-3.08 (m, 2H, ^{3}H5'), 3.53, 3.56 (two s, 6H, 2 x 2'-OMe), 3.71-3.81 (m, 3H, ^{3}H2', ^{5}H2' and ^{5}H4'), 3.91-4.22 (m, 4H, ^{3}H4', ^{5}H5' and ^{3}H3'), 5.71 (d, 1H, ^{3}H5), 5.86 (s, 2H, ^{3}H1' and ^{5}H1'), 7.72-7.66 (m, 11H, Ph, ^{5}H6 and ^{3}H6), 9.91, 10.09 (two br s, 2H, 2 x NH), $J_{5H1'-5H2'}$ = 0, $J_{3H1'-3H2'}$ = 0, $J_{3H5-3H6}$ = 8.0; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 11.93 ppm (^{5}5-Me),

19.46 (**C**Me$_3$), 24.08 (^{5}C1"), 27.00 (C**Me**$_3$), 31.48 (^{5}C2"), 33.28 (^{3}C5'), 39.82 (^{5}C3'), 58.07 and 58.78 (2 x 2'-OMe), 62.49 (^{5}C5'), 70.99 (^{3}C3'), 82.33, 83.21 (2 x C2'), 84.87, 85.15 (2 x C4'), 88.13, 88.67 (2 x C1'), 102.63, 110.39 (2 x C5), 127.87-139.78 (Ph and 2 x C6), 150.05, 150.41 (2 x C2), 164.01, 164.34 (2 x C4); MS (FAB-NBA) m/e 795.6 ([MH$^+$], 13.9%), 669 ([MH$^+$ - ThyH], 100), 521 (16.9), 369 (23.1), 315 (54.3); HRMS (FAB-Glycerol) m/e calcd. for $C_{39}H_{50}N_4O_{10}SSi$ + H$^+$: 795.30952, found: 795.30957.

Diol 33

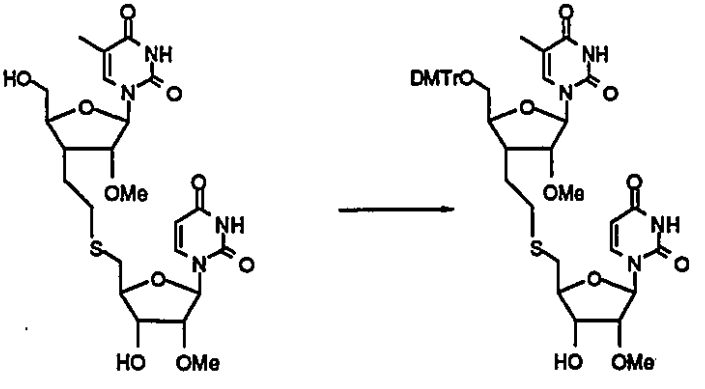


32 **33**

A solution of tetra-*n*-butylammonium fluoride in THF (1M, 0.22 ml, 0.22 mmole) was added to a solution of dimer **25** (90 mg, 0.11 mmole) in dry THF (0.75 ml). After 3 h the solution was evaporated and the resulting syrup was chromatographyed over silica gel (20:1 CH_2Cl_2 / MeOH, v/v) giving diol **26** (51 mg, 81%), m.p. 148-151^{0}C (CH_2Cl_2 / hex): ^{1}H-NMR (200 MHz, CD_3OD) δ 1.43-1.68 (m, 2H, ^{5}H1"), 1.74 (s, 3H, ^{5}5-Me), 2.18 (H^7, 1H, ^{5}H3'), 2.40-2.58 (m, 2H, ^{5}H2"), 2.72-2.78 (m, 2H, ^{3}H5'), 3.41 (s, 6H, 2 x 2'-OMe), 3.59-3.70 (m, 3H, ^{3}H2', ^{5}H2' and ^{5}H4'), 3.82-3.96 (m, 4H, ^{3}H4', ^{5}H5'

and ^{3}H3'), 5.61 (d, 1H, ^{3}H5), 5.72 (d, 1H, ^{3}H1') 5.74 (s, 1H, ^{5}H1'), 7.53 (d, 1H, ^{3}H6), 7.99 (s, 1H, ^{5}H6); $J_{3H5-3H6}$ = 8.0; ^{13}C-NMR (55.5 MHz, CD_3OD) δ 12.49 ppm (^{5}5-Me), 24.32 (^{5}C1"), 31.66 (^{5}C2"), 33.66 (^{3}C5'), 39.19 (^{5}C3'), 58.22, 58.95 (2 x 2'-OMe), 59.99 (^{5}C5'), 71.34 (^{3}C3'), 82.75, 83.52 (2 x C2'), 85.71, 85.90 (2 x C4'), 88.70, 88.82 (2 x C1'), 102.70, 110.01 (2 x C5), 137.07, 140.80 (2 x C6), 150.62, 150.91 (2 x C2), 164.60, 165.35 (2 x C4); MS (FAB-NBA) m/e 557 ([MH^+], 26.9%), 431 ([MH^+ - ThyH], 44.9), 307 (31.2), 195 (40.4), 186 (100), 128 ([ThyH + $2H^+$], 100), 113 ([UraH + H^+], 30.4); HRMS (FAB-Glycerol) calcd. for $C_{23}H_{32}N_4O_{10}S + H^+$: 557.19174, found, 557.19149.

Dimethoxyltrityl ether 34

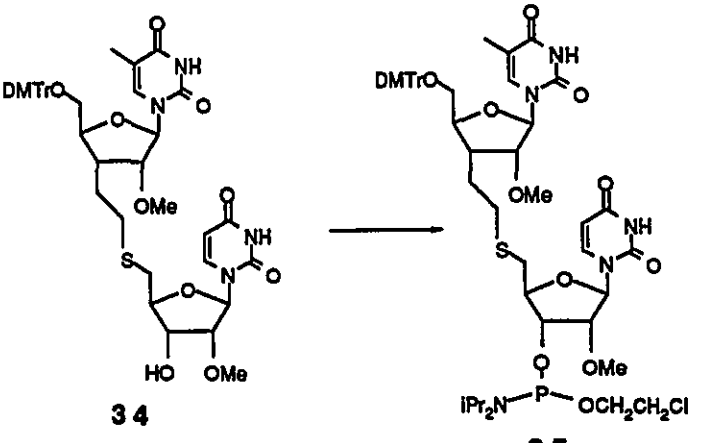


33 **34**

4,4-Dimethoxytrityl chloride (80 mg, 0.24 mmole) was added to a solution of diol **33** (44 mg, 0.08 mmole) in dry pyridine (1.10 ml) and triethylamine (0.06 ml, 0.39 mmole) at room temperature. After 10 h the solvent was removed and the resulting syrup was chromatographyed over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) giving **34** (56 mg, 83%), m.p.

84-86^{0}C (EtOH /H_2O): ^{1}H-NMR (200 MHz, acetone) δ 1.40 (m, 2H, ^{5}H1"), 2.02 (s, 3H, ^{5}5-Me), 2.58-2.94 (m, 5H, ^{3}H5', ^{5}H3' and ^{5}H2"), 3.24 (m, 1H, ^{5}H2'), 3.48 and 3.61 (two s, 6H, 2 x 2'-OMe), 3.65 (m, 1H, ^{5}H5'$_A$), 3.79 (s, 6H, PhOMe), 3.92-4.31 (m, 4H, ^{3}H4', ^{3}H3' ^{5}H4' and ^{5}H5'$_B$), 5.56 (d, 1H, ^{3}H5), 5.86 (s, 1H, ^{5}H1'), 5.89 (d, 1H, ^{3}H1'), 6.85-7.51 (m, 9H, Ph), 7.68 (d, 1H, ^{3}H6), 7.81 (s, 1H, ^{5}H6), 10.17 (br s, 2H, 2 x NH), $J_{5H1'-5H2'}$ = 0, $J_{3H1'-3H2'}$ = 5.1, $J_{3H5-3H6}$ = 8.2; ^{13}C-NMR (55.5 MHz, acetone) δ 11.92 ppm (^{5}5-Me), 26.18 (^{5}C1"), 30.69 (^{5}C2"), 34.84 (^{3}C5'), 39.81 (^{5}C3'), 40.97 (^{3}C2'), 46.34 (Ph_3**C**), 55.49 (PhOMe), 58.45 (2 x 2'-OMe), 62.60 (^{5}C5'), 73.91 (^{3}C3'), 84.65, 85.15, 86.31 (^{5}C2', ^{3}C1' and 2 x C4'), 87.15 (^{5}C1'), 109.99, 110.90 (2 x C5), 127.20-145.56 (Ph and 2 x C6), 151.05, 151.21 (2 x C2), 159.67, 164.36 (2 x C4); MS (FAB-NBA) m/e 944 ([M + Et_3NH^+], 4.7 %), 842 ([M], 2.0), 460 (10.1), 391 (16.5), 303 ([DMTrH], 100); HRMS (FAB-Glycerol) m/e calcd. for $C_{44}H_{50}N_4O_{12}S$ + H^+: 859.32242, found: 859.32257.

Amidite 35



34 35

2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.100 mL, 0.45 mmol) was slowly added to a stirred solution of tritylated alcohol **34** (195 mg, 0.22 mmol) in dry dichloromethane (1 mL) containing triethylamine (0.077 mL, 0.52 mmol). After 14 h of stirring at room temperature under nitrogen, the solvent was evaporated yielding a pale yellow foam. The crude product was chromatographyed over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) affording the phosphoramidite **35** as a colorless foam (205 mg, 86%), which was used as such in the subsequent solid-phase syntheses: ^{1}H-NMR (200 MHz, acetone) δ 1.08-1.25 (m, 12H, -N(CH**Me**$_2$)$_2$) 1.42 (m, 1H, ^{5}H1$_A$"), 1.83 (m, 1H, ^{5}H1$_B$"), 2.07 (s, 3H, 5-Me), 2.58-2.95 (m, 9H, ^{5}H3', ^{3}H2', ^{5}H2", ^{3}H5' and -CH_2CN), 3.26 (m, 1H, 5H2'), 3.52 and 3.55 (2s, 6H, 2 x 2-OMe), 3.82 (s, 6h, 2 x PhOMe), 3.60-4.43 (m, 9H, ^{5}H5',^{3}H4', ^{5}H4', ^{3}H3', -N(**CH**$_2$Me$_2$)$_2$ and -POCH$_2$), 5.80 (m, 1H, ^{5}H1'), 6.97 (m, 1H, ^{3}H1'), 6.90-7.81 (m, 15H, DMTr and 2 x H6), 10.08 (s, 2H, 2 x NH); ^{31}P-NMR (81.0 MHz, acetone) δ 152.61 and 152.27 ppm; MS (FAB - Glycerol / NBA) m/e 1059 ([MH$^+$], 12.8%), 988 (19.0), 933 ([MH$^+$ - ThyH], 7.2), 841 ([MH+ - *i*Pr$_2$NH$^+$P(OH)OCH$_2$CH$_2$CN], 5.5), 689 (18.8), 474 (100).

Experimental for Chapter 5

Solid-phase synthesis of oligonucleotides

All reagents used in the solid-phase synthesis were purchased from Pharmacia. Deoxyribonucleoside phosphoramidites with a benzoyl group as the protecting function were also obtained from Pharmacia and were used as 0.1 M acetonitrile solutions. Each of four activated sulfide dimers, which were dimer **9**, **21**, **A** and **35** described above, was used as a 0.11 M acetonitrile solution. Synthesis of all oligomers was carried out on a Pharmacia GLB Gene Assembly Plus synthesizer. Each sequence synthesis started from the 3'- to the 5'-end, and the solid-supports bearing a 3'-terminus deoxyribonucleoside with the bases thymine and N^4-benzoylcytosine were obtained from Pharmacia. All sequences were prepared on a 0.2 μmole scale and "trityl off" mode employing the standard DNA synthesis cycle provide by Pharmacia except for the coupling time for the incorporation of a sulfide dimer, which was extended to 9 min. The sequences prepared were listed in Figure 31. The average coupling efficiencies for oligomers **36-49**, which were automatically measured by the synthesizer, were between 78-100% (Table 6).

Cleavage, Deprotection and Desalting of Oligonucleotides

A oligo-support cassette removed from synthesizer was placed in a 2 ml Eppendorf tube and centrifuged to get rid of any trapped acetonitrile. Then this cassette was transferred to a new Eppendorf tube and treated with 1.00 ml of a 29% ammonium hydroxide solution. After incubatation at 55^0C for 16 h, the deprotection and the cleavage of this oligomer from the solid-support were completed. The ammonia solution which contained the

	sequence	
(36)	5'- G C G T T T T^{OH}_{s} T T T G C T	-3'
(37)	5'- G C G T T^{OH}_{s} T T T T T^{OH}_{s} T G C T	-3'
(38)	5'- G C G T T^{OH}_{s} T T T^{OH}_{s} T T T^{OH}_{s} T G C T	-3'
(39)	5'- G C G T T T T^{OMe}_{s} T T T G C T	-3'
(40)	5'- G C G T T^{OMe}_{s} T T T T T^{OMe}_{s} T G C T	-3'
(41)	5'- G C G T T^{OMe}_{s} T T T^{OMe}_{s} T T T^{OMe}_{s} T G C T	-3'
(42)	5'- G C G T T $T_{s}U^{OMe}$ T T G C T	-3'
(43)	5'- G C G $T_{s}U^{OMe}$ T T $T_{s}U^{OMe}$ G C T	-3'
(44)	5'- G C G $T_{s}U^{OMe}$ $T_{s}U^{OMe}$ $T_{s}U^{OMe}$ G C T	-3'
(45)	5'- G C G T T $T^{OMe}_{s}U^{OMe}$ T T G C T	-3'
(46)	5'- G C G $T^{OMe}_{s}U^{OMe}$ T T $T^{OMe}_{s}U^{OMe}$ G C T	-3'
(47)	5'- G C G $T^{OMe}_{s}U^{OMe}$ $T^{OMe}_{s}U^{OMe}$ $T^{OMe}_{s}U^{OMe}$ G C T	-3'
(48)	5'- G C G T T T T T T G C T	-3' (DNA)
(49)	5'- A G C A A A A A A C G C	-3' (DNA)

Figure 31 Prepared oligomers **36-49** are shown in the 5' to 3' direction. $T^{OH}_{s}T$ denotes the ^{5}2'-OH sulfide-linked dimer (**9**); $T^{OMe}_{s}T$ represents the ^{5}2'-OMe sulfide-linked dimer (**21**); $T_{s}U^{OMe}$ stands for the ^{3}2'-OMe sulfide linked dimer (**A**), and $T^{OMe}_{s}U^{OMe}$ for the di-^{5,3}2'-OMe sulfide-linked dimer (**35**).

Table 6 Average coupling efficiencies and concentrations of the oligomers.

sequence	36	37	38	39	40	41	42	43	44	45	46	47	48	49
efficiency	88.7	88.9	77.7	100	93.9	80.0	100	100	100	100	100	91.5	100	100
OD/ml	6.9	5.8	4.4	7.9	3.0	2.2	9.7	12.1	8.0	6.6	4.4	4.4	7.2	8.4

oligomer prepared was collected by centrifuging the Eppendorf tube and was run though a Sephadex NAP-10 column which was pre-washed with 15 ml of deionized water. 1.50 ml of deionized water was used as eluent and the eluent was collected as soon as the water was added. The eluting solvent was lyophilized to dryness and the resulting oligomer was redissolved in 1.00 ml of water. Purity of each oligomer was identified by PAGE (26% polyacrylamide) which was run at 4^0C for 6-8 h at a current of 10 mA. About 0.3 A_{260}[93] unit was applied in the PAGE. Following electrophoresis, the gels were wrapped in plastic wrap (Saran Wrap^{Tm}), placed over a fluorescent TLC plate and illuminated with a UV lamp (254 nm). The gels were photographed using Polaroid PolaPlanTm 4X5 cm Instant Sheet Film (#52, medium contrast, ISO 400/27^0) through a Kodak Wratten gelatin filter (#58 green). The single absorption band on polyacrylamide gel found for all oligomers indicated their high purity.

Tm measurements

Thermal denaturation were performed on a Varian-Cary 1 UV/vis spectrophotometer equipped with a Peltier thermal unit and a six cells transport mechanism. Extinction coefficients for the sequences 1 and 2 were 105.08 $cm^{-1}mol^{-1}$ and 128.34 $cm^{-1}mol^{-1}$, which were calculated using the nearest-neighbour approximation[94]. For modified oligomers 3-15 containing sulfide links, extinction coefficients were assumed to be the same as that of its analogue 1. Samples for Tm measurement were prepared by mixing 2.5 µmole of each complementary strand, lyophilizing

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93 An A260 unit is the amount of material that will produce an absorbance of 1.0 at 260 nm, when dissoved in 1.0 ml of water, in a 1-cm cell.

94 Puglisi, J. D.; Tinoco, jr. I.: *Methods in Enzymology*, **180**, 304 (1989).

the solvent to dryness and dissolving in 1.00 ml of 1.0 M sodium phosphate buffer solution[95], except for the measurements of sequence 5 with its complementary DNA and RNA, which were also analysized in 10 mM, 100 mM, 210 mM and 910 mM sodium phosphate buffer solutions. The oligonucleotide mixture (2.5 μmole in each strand in 1.0 M buffer) was added to a 1 cm path length - 1.5ml quartz cuvet and placed in the spectrophotometer which was set on the dual beam optical mode to reduce optical drift. The cell were equilibrated at 40^{0}C for 10 min and slowly cooled to the start temperature over a 30 min period. Thermal denaturation curves were obtained by recording the absorbance (average of 30 readings) at 260nm at 1 min intervals as the temperature was ramped to 80^{0}C at 0.5^{0}C/min. This resulted in approximately 140 points per thermal curve.

Tm values were reported as the interaction of the absorbance versus temperature curves and the midpoint between the upper and lower linear sloping base lines.

Native-gel Hybridization

A 24% polyacrylamide gel containing no urea was pre-incubated in 0.09 tris-borate-EDTA running buffer (pH 8.3) for 1 h at 4^{0}C using 1 mA applied current. Mixtures containing **38/49, 38/50, 48/49** and **48/50** (all single strand was used in 3 μmole) were each dissolved in 7 μl of 30% sucrose/10 mM $MgCl_2$ and incubated initially at 40^{0}C for 30 min and then at 20^{0}C for 20 min. Each sample was applied to a separate lane of the gel.

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95 1.0 M sodium phosphate buffer solution was prepared by adding NaCl crystals (5.8 g) to NaH_2PO_4/Na_2HPO_4 (85.7 mg/53.7 mg, ca. pH 7) in deionized/autoclaved water and adjusted to pH 7.0 using HCl/NaOH.

Electrophoresis was conducted at 4^0C for 18 h at a constant current of 5 mA. Following electrophoresis, the gel was photographed as described above.

Experimental for Chapter 6

Preparation of Sulfone-linked Oligonucleotides

To a Eppendorf which contained only one sulfide-linkage oligomer was added methanol (4 µl/1 OD oligomer) and aqueous oxone stock (0.06 mmol/ml, 5 µl/1 OD oligomer). For the oligomers which contained two or three sulfide-linkage dimers in the sequence, the amounts of methanol and oxone stock used was increased by a factor of 2 or 3. The resulting solution was kept at room temperature for 16 h and then was diluted with deionized/autoclaved water to 1.0 ml. The following desalting proceeding was the same as described above. The purified sulfone-containing oligomers were obtained as aqueous solutions and quantified at 260 nm (Table 7).

Table 7 Concentrations of sulfone-containing oligomers.

	51	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**
OD/ml	1.7	0.9	0.7	1.5	0.7	0.5	1.6	1.0	1.9	1.0	0.7	0.8

Identification of Sulfone-containing Oligomer

Oligomer **53** (0.3 OD) obtained above was treated with 29% ammonium hydroxide (75 µl) at 55^{0}C for 2 h. The solvent was lyophilized to dryness, redissolved in 80% DMF (10 µl) and applied to a lane of the gel. Oligomer **38** (0.3 OD) which was obtained by cleaving from the solid-support with 29% ammonium hydroxide at 55^{0}C for 16 h, and **53** (0.3 OD)

were also applied on the same gel in separate lanes. Following electrophoresis, the gel was photographed as described above.

Tm Measurements

The Tm values were measured using the same method described above, in 1.0 M NaCl, pH 7.0 NaH_2PO_4-Na_2HPO_4 buffer solution.