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The Stereoselective Synthesis of Antisense Phosphorothioates and Boranophosphates

By
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A Thesis Submitted to the Faculty of Graduate Studies and Research in Partial Fulfillment of the Requirements of the Degree of

Doctor of Philosophy

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In Memory of My Parents.

To My Loving Husband and Colleague,

Fu Chen

For My Beloved Daughters,

Jinxi Chen

and Mary Jinping Chen

ABSTRACT

Chiral auxiliary 1,2-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **51** and its enantiomer **52** were synthesized from D-xylose and L-xylose respectively and treated with PCl_3 to form cyclic phosphochloridites **58** and **67**, and then reacted with 5'-O-*t*-butyldimethylsilylthymidine to give diastereomerically pure cyclic phosphoramidites **60** and **68**. They were coupled with 3'-O-*t*-butyldimethylsilylthymidine in the presence of 2-bromo-4,5-dicyanoimidazole **46** as catalyst, and then sulfurized with Beaucage's reagent to form protected dithymidine phosphorothioates **62** and **72** stereoselectively. After deprotection, Sp and Rp dithymidine phosphorothioates **65** and **66** were obtained respectively in 98% diastereomeric excess. The mechanism of the coupling reaction which involves only one inversion in the transformation of the phosphoramidites (**60**, **68**) to the dithymidine phosphorothioates (**65**, **66**) is discussed. The effect of the acidity of the catalyst on the stereoselectivity and the rate of the coupling reaction was studied.

The synthesis and separation of diastereomers of protected dithymidine boranophosphates Sp-**107** and Rp-**108** gave the dimers Sp-**109** and Rp-**110** in a stereospecific manner. These dimers will be incorporated into oligonucleotides to synthesize mixed backbone oligonucleotides. To confirm the stereochemistry of the dimers Sp-**109** and Rp-**110**, the free dithymidine boranophosphates Sp-**115** and Rp-**116** were synthesized, the configurations at the phosphorus atom of which were assigned by comparison with spectral data from the literature.

A stereoselective synthesis of a dinucleotide boranophosphate Sp-**115** with a de of > 98%, using (S)-3-hydroxyl-4-(2-indolyl)butyronitrile **28**-(S) as chiral auxiliary, is described. The conversion of phosphite triester to boranophosphate by BH_3-Me_2S proceeds with retention of configuration at the phosphorus atom. The procedure may be adaptable to the solid phase synthesis.

RESUME

L'auxiliaire chiral 1,2-O-cyclopentylidène-5-désoxy-5-isopropylamino-α-D-xylofuranose **51** et son énantiomère **52** ont été synthétisés à partir du D-xylose et L-xylose respectivement, puis traités avec du PCl_3 pour former les phosphochloridite cycliques **58** et **67**. Celles-ci ont alors été mises en réaction avec la 5'-O-*t*-butyldiméthylsilylthymidine pour donner les phosphoramidites cycliques diastéréoisomériquement pures **60** et **68**. Le couplage avec la 3'-O-*t*-butyldiméthylsilylthymidine en présence de 2-bromo-4,5-dicyanoimidazole **46** comme catalyseur, suivi de la sulfurization par le réactif de Beaucage a permis la formation stéréosélective des dithymidines phosphorothioates protégés **62** et **72**. Après déprotection, les dithymidines phosphorothioates Sp-**65** et Rp-**66** sont obtenus avec un excès diastéréoisomérique de 98%. Le mécanisme de la réaction de couplage conduisant à une seule inversion de configuration lors de la transformation des phosphoramidites (**60**, **68**) en dithymidines phosphorothioates (**65**, **66**) a fait l'objet d'une discussion. L'effet de l'acidité du catalyseur sur la stéréosélectivité et le taux de couplage a été étudié.

La synthèse et la séparation des diastéréoisomères Sp-**107** et Rp-**108** de dithymidines boranophosphates protégés a conduit aux dimères Sp-**109** et Rp-**110** de manière stéréospécifique. Ces modimères seront incorporés au sein d'oligonucléotides pour synthétiser des oligonucléotides à squelette varié. Pour confirmer la stéréochimie des dimères Sp-**109** et Rp-**110**, les dithymidines boranophosphates Sp-**115** et Rp-**116** ont été synthétisées. Les configurations de l'atome de phosphore ont été attribuées par comparaison avec les données spectrales de la littérature.

Une synthèse stéréosélective de dinucléotide boranophosphate Sp-**115** avec un ed > 98%, utilisant le (S)-3-hydroxyl-4-(2-indolyl)butyronitrile **28**-(S) comme auxiliaire chiral, est également décrite. La conversion du phosphite triester en boranophosphate BH_3-Me_2S se produit avec rétention de configuration sur l'atome de phosphore. La procédure pourrait être adaptée à la synthèse sur support solide.

ACKNOWLEDGMENTS

I am deeply grateful to my supervisor, Professor George Just, for his invaluable guidance, unwavering support, great patience and encouragement during the course of my studies and research.

I am grateful to the Chemistry Department and to McGill University for funding in the form of a teaching assistantship and of a J. W. McConnell McGill Major Fellowship, an Alexander McFee Award and a Beijing Memorial McGill Major Fellowship. I thank FCAR for funding in the form of a FCAR Graduate Fellowship. My appreciation is also extended to NSERC, ISIS Pharmaceuticals (Carlsbad, CA) and Dalton Chemical Laboratories (Toronto) for generous financial support.

I wish to thank my husband Fu Chen for his love, support, encouragement and sharing of success and failure throughout my thesis work.

I would also like to thank:

Mr. Ulhas Bhatt, Mr. Giancarlo Biancotto, Dr. Jaffar A. Damavandi, Mr. Kevin Leung, Mr. Yixin Lu, Dr. Eric Marsault, Dr. Nazim Mohamed, Dr. Marija Prhavc, Dr. Jianchao Wang, Dr. Constantin Yannopoulos, Dr. Wenqiang Zhou, as well as all my past and present co-workers in rooms 237-238 for their friendship and helpful advice and discussions.

Dr. Arlène Roland for translation of the abstract.

Dr. Françoise Sauriol for her ever-cheerful assistance in NMR experiments.

Dr. Orval Mamer, Mr. Nadim Saadeh and Dr. Daniel Boismenu for the recording of mass spectra.

Ms. Renee Charron for her invaluable help in administrative matters.

Special thanks to:

Mr. Ulhas Bhatt, Mr. Kevin Leung, Mr. Yixin Lu, Dr. Nazim Mohamed and Dr. Arlène Roland for proof-reading this manuscript.

My friends: Dr. Jiahua Chen, Ms. Xiaoling Gao, Ms. Wenshuo Lu, Mr. Yongchong Luo, Ms. He Xiao, Ms. Linghua Yu, Dr. Xiaoming Zhang and Ms. Lihua Zheng for their friendship and help to make my life easy and enjoyable in Montreal.

All the people in Dr. Chan's lab, Dr. Chin's lab, Dr. Damha's lab, Dr. Hay's lab, Dr. Kazlauskas's lab for their help in using their equipment.

GLOSSARY OF ABBREVIATIONS

A	adenine
Ac	acetyl
AIDS	acquired immuno-deficiency syndrome
AOT	antisense oligonucleotides
Ar	aryl
Ar	argon
atm	atmosphere
b	broad (NMR)
B	base
BDT	1,3-benzodithiol-2-yl
BNCT	boron neutron capture therapy
b.p.	boiling point
BSA	N,O-bis(trimethylsilyl)acetamide
BSPDE	calf spleen phosphodiesterase
Bu	*n*-butyl
c	concentration (for the measurement of optical rotation)
C	cytosine
C	Celsius
calcd	calculated
CI	chemical ionization
COSY	correlation spectroscopy
CPG	controlled pore glass
CpsT	3'-Cytosine-5'- thymidine phosphorothioate
δ	chemical shift (NMR)
d	doublet (in NMR)
dA	2'-deoxyadenosine
DBU	1,8-diazabicyclo[5,4,0]undec-1-ene

DCC	dicyclohexylcarbodiimide
DDS	isodurenedisulfonyl dichloride
de	diastereomeric excess
DECP	diethyl phosphorochloridate
DIAD	diisopropyl azodicarboxylate
DMAP	N,N-dimethyl-4-aminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMT or DMTr	dimethoxytrityl (4,4'-dimethoxytriphenylmethyl)
DNA	deoxyribonucleic acid
dT	2'-deoxythymidine
d(TpBT)	dithymidine boranophosphosphate
E. coli:	*Escherichia Coli*
EDTA	ethylenediaminetetraacetic acid
EI	electron ionization
eq.	equivalent(s)
Et	ethyl
EtOAc	ethyl acetate
FAB	fast atom bombardment
g	gram(s)
G	guanine
GMA	good manufacture analysis
h	hour(s)
HCMV	cytomegalovirus (CMV) in HIV patient
HETCOR	hetero correlation
HIV	human immunodeficiency virus
HMQC	heteronuclear multiple quantum correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	Hertz

ICAM-1	intercellular adhesion molecular 1
IDTr	3-(imidazol-1-ylmethyl)-4,4'-dimethoxytrityl
J	coupling constant (NMR)
lit.	literature
m	multiplet (NMR)
Me	methyl
m/e	mass-to-charge ratio
mg	milligram(s)
MHz	megaHertz
min.	minute(s)
MMI	methylene(methylimino)
mmol	millimole(s)
ml or mL	milliliter(s)
mol	mole(s)
MMTr	monomethoxytrityl (4-methoxyphenyl)diphenylmethyl)
m.p.	melting point
mRNA	*messenger* ribonucleic acid
Ms	mesyl (methanesulfonyl)
MS	mass spectrometry (spectrum)
N	normality (solution)
NBA	nitrobenzyl alcohol
nBu	n-butyl
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
ODN	oligodeoxynucleotide
ONA	peptide nucleic acids containing ornithine
p-	para
PAO-TMG	tetramethylguanidinium 2-pyridinaldoximate
PKC	protein kinase C
Ph	phenyl

PNAs	peptide nucleic acids
ppm	parts per million
PSI	pounds per square inch (1 psi = 0.06804 atm)
PS-Oligos	phosphorothioate oligonucleotides
q	quartet (NMR)
R_f	retardation factor
RNA	ribonucleic acid
RNase H	ribonuclease H
RT (rt)	room temperature
RP	reverse phase
s	singlet (NMR)
sec.	second(s)
SVPDE	snake venom phosphodiesterase
t	triplet (NMR)
T	thymine
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
tBu	*tet*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
T_3-OH	5'-O-protected-thymidine
T_5-OH	3'-O-protected-thymidine
TPS	3-trimethylsilylpropionate-2,3,3,3-d_4-sodium salt
TpsT	dithymidine phosphorothioate
Tr	trityl (triphenylmethyl)
tRNA	*transfer* ribonucleic acid

Ts	tosyl (*para*-toluenesulfonyl)
µl	microliters
µmol	micromoles
U	uracil
UV	ultraviolet
v	volume
w	weight

TABLE OF CONTENTS

Chapter 1

Introduction & Literature Review

Chapter 2

The Stereoselective Synthesis of Phosphorothioates

Chapter 3

The Chemical Synthesis of Sp and Rp Diastereomers of Dithymidine Boranophosphates

Chapter 4

The Stereoselective Synthesis of Dinucleotide Boranophosphates

"For a successful technology, reality must take precedence over public relations, for nature cannot be fooled."

Richard Feynmann

Nobel Laureate, **1965**.

Chapter 1

Introduction & Literature Review

"Molecules that bind with specific messenger RNAs can selectively turn off genes. Eventually certain diseases may be treated with them; today antisense molecules are valuable research tools."

Harold M. Weintraub, *Scientific American*, **1990**, *262*.

1.1. Antisense Strategy

1.1.1. What Is Antisense Strategy?

(1) Proteins - The Building Blocks of Life

Proteins composed of aminoacids are the basic building blocks of life. They maintain the structural integrity of cells and organs and serve as catalysts for biological reactions (enzymes). Many diseases are associated with defective protein production.

The information necessary to produce proteins in cells is contained in genes. Specific genes contain information to produce specific proteins. The information required for the human body to produce all proteins is contained in the human genome and its collection of more than 100,000 genes. Genes are made up of DNA which contains information about when and how much of which protein to produce, depending upon what function is to be performed.

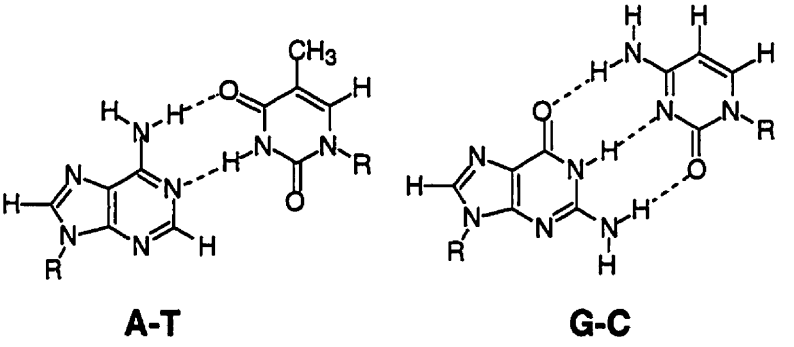


Figure 1.1: Watson-Crick base-pairing interactions

The DNA molecule is a "double helix" - a duplex of entwined strands. In each duplex, the bases or nucleotides (Adenine, Thymidine, Guanine, Cytosine) are weakly bound or "paired" by hydrogen bonds to complementary nucleotides on the other strand (A to T, G to C), i.e. Watson-Crick base pairing interactions[1] (Figure 1.1). Such highly specific complementary base pairing is the essence of information transfer from DNA to its

intermediary, messenger RNA (mRNA) which carries the information, spelled out by the specific sequences of bases, necessary for the cell to produce a specific protein.

During transcription of information from DNA into mRNA, the two complementary strands of the DNA partly uncoil. The "sense" strand separates from the "antisense" strand. The "antisense" strand of DNA is used as a template for transcribing enzymes which assemble mRNA - a process called "transcription". Then, mRNA migrates into the cell where other cellular structures called ribosomes read the encoded information, its mRNA's base sequence and in so doing, string together amino acids to form a specific protein. This process is called "translation"[2] (Figure 1.2).

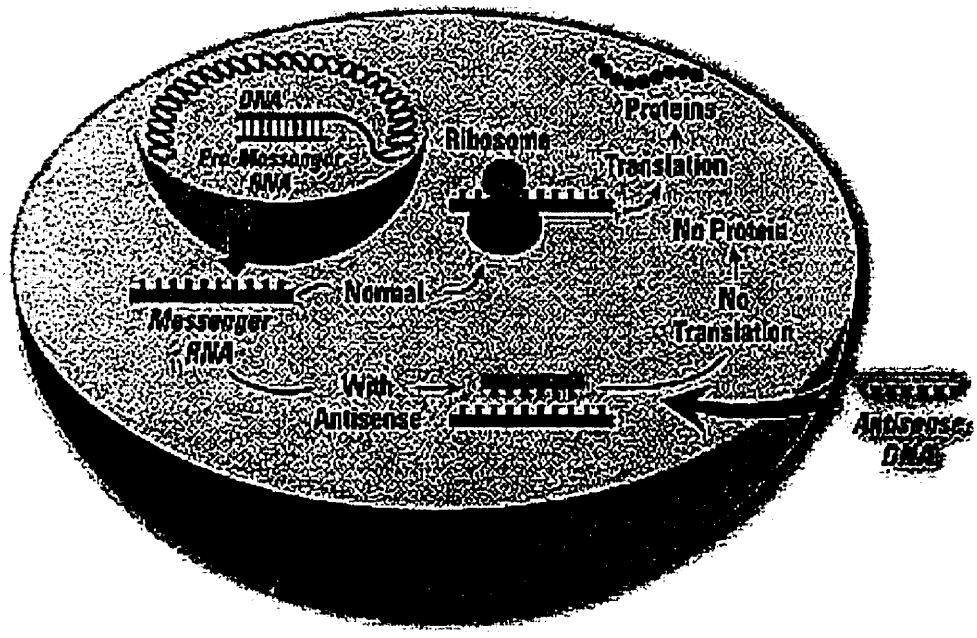


Figure 1.2: Gene Expression and Antisense Action[3]

(2) Antisense Strategy

Antisense molecules are synthetic segments of DNA or RNA ("oligonucleotides"), designed to mirror specific mRNA sequences and block protein production. Each antisense drug is designed to bind to a specific sequence of nucleotides in its mRNA target to inhibit production of the protein encoded by the target mRNA (Figure 1.2).

The concept of using short segments of synthetic DNA as specific inhibitors of gene expression was proposed by Grineva in 1967[4] and based in part by the observation that RNA sequences serve as endogenous biological inhibitors of gene expression in prokaryotes.[5] In 1978, Zamecnik and Stephenson[6,7] first described the inhibition of Rous sarcoma virus replication and of RNA translation by synthetic oligonucleotides. Since then, antisense strategy has been widely studied. Advances in chemistry and molecular biology have provided the basis to develop antisense oligonucleotides and improve their selectivity, stability and specificity of action. The antisense technology has been extensively used *in vitro* and *in vivo* as a tool to study the regulatory mechanisms in biological processes and as potential therapeutics in cancer, viral infections and genetic disorders.[8]

By acting at the translation stage in the disease-causing process to prevent the production of a disease-causing protein, antisense drugs have the potential to provide greater therapeutic benefits than traditional drugs which do not act until the disease-causing protein has already been produced.

Antisense drugs have the potential to be much more selective or specific than traditional drugs, and therefore more effective with fewer side effects.

Finally, one of the major attractions of the antisense approach is that it allows the rational design of drugs. The design of antisense compounds are less complex, more rapid and more efficient than traditional drug design directed at protein targets. The traditional drug design usually begins by characterizing the three-dimensional structure of the protein target in order to design a prototype drug to interact with the target. Proteins, however, are complex molecules whose structures are difficult to predict. In contrast, antisense compounds are designed to bind to mRNA whose structures are more easily understood and predicted. Once the receptor sequence on the mRNA is identified, the three-dimensional structure of the receptor site can be defined, and the prototype antisense drugs can be designed.

1.1.2. The Mechanisms of Antisense Action

There are multiple theoretical mechanisms by which oligonucleotides can be used to regulate expression of target genes.[9,10,11]

It turned out that one of the main factors responsible for the efficacy of oligodeoxynucleotides at inhibiting mRNA translation was the cleavage of the mRNA in its DNA hybrid by a ribonuclease called RNase H.[12,13] This enzyme recognizes DNA-RNA hybrids and cleaves only the RNA strand. When the oligonucleotide is targeted to the coding sequence of the mRNA, inhibition is observed only when the mRNA is cleaved by RNase H in the hybrid. The translating ribosomes are endowed with an unwinding activity which releases any oligonucleotide hybridized to the coding sequence. Of course, RNase H-induced cleavage of the mRNA stops the transcription process. So far, this is the most widely observed mechanism in antisense action. Although it has not been unequivocally demonstrated that reduction or cleavage of the targeted RNA in cells is mediated by RNase H, there is a great deal of evidence to support such a conclusion, including direct demonstration of a reduction in target mRNA, demonstration of appropriate cleavage products,[14,15,16] and use of modified oligonucleotides that do not support RNase H activity.[17,18,19]

Translation arrest is one of the most cited mechanisms in antisense action, in which the oligonucleotide binds to the target mRNA and blocks movement of the ribosome and subsequently translation of the mRNA. Reduction in targeted protein by an oligonucleotide but no reduction in mRNA has been used as evidence for a translation arrest mechanism. Alternatively, demonstration of a selective reduction in target protein by modified oligonucleotides that do not support RNase H has also been used as evidence for a translation arrest mechanism. However, in both cases other mechanisms of action could account for these observations. Therefore, evidence directly demonstrating a translation arrest mechanism in cell culture or *in vivo* is circumstantial.[20]

A somewhat related mechanism is the prevention of ribosome assembly on the mRNA. Baker *et al.*[21] utilized uniformly 2'-modified oligonucleotides, which do not support RNase H activity, to target the 5'-terminus of ICAM-1 RNA. These oligonucleotides very effectively inhibited ICAM-1 protein expression by markedly changing the polysome profile of ICAM-1 mRNA, shifting it from a higher molecular weight polysome pool to a lower molecular weight pool.

Regulating pre-mRNA maturation is also a potential mechanism by which oligonucleotides may inhibit or alter gene expression by sterically blocking recognition of the RNA.[22,23]

These results demonstrate that there are multiple antisense mechanisms by which oligonucleotides can inhibit expression of genes. The mechanism of action is dependent in part on where the oligonucleotide hybridizes on the RNA, as well as the type of oligonucleotides used. The effectiveness of the antisense mechanism continues to be proven in the laboratory, in animal studies and in human trials.

1.1.3. The Requirements for Antisense Drugs

To be effective as antisense drugs, antisense oligonucleotides must fulfill several requirements.[24]

(1) Oligonucleotide Stability

Antisense drugs have to be resistant to intracellular and extracellular enzymes. Otherwise, they would be degraded before having time to perform their therapeutic activity.

(2) Oligonucleotide Length

The optimal length of an antisense oligonucleotide is based in part on the statistical calculation that a particular sequence of 13 bases in RNA and of 17 bases in DNA should be found only once in the entire human genome, thus representing unique elements within

the cell.[25] Therefore, in theory an oligonucleotide 11 - 15 bases in length should unique hybridize to a given mRNA, while an oligonucleotide 15 - 19 bases in length should hybridize to a unique DNA sequence depending on the A + T and G + C content. Shorter than 15 bases increases chances of cross-hybridization to other mRNAs. Longer than 25 bases may decrease uptake and efficacy.[26]

(3) Cellular Uptake

Antisense drugs need to be internalized by the cells involved in a particular pathological process. Furthermore, inside the cell a significant proportion of the oligonucleotide must be available in the same cellular compartment as the target RNA to allow hybridization to occur.

(4) Target Sequence Selection

Ideally, inhibition of protein synthesis should be completely selective and leave expression of other proteins unaffected. Furthermore, it is desirable that the interaction of antisense molecules with other molecules, e.g., proteins, carbohydrate moieties, lipids, second messenger molecules, and so forth, does not lead to adverse biological effects.

(5) Purity of compound, Homogeneity of Product, and Low Toxicity

Like any other drugs, antisense pharmaceuticals need to meet certain standards regarding purity and homogeneity of the compound and the definition of GMA standards. Furthermore, toxic side effects need to be as low as possible.

1.1.4. Chemical Modifications of Antisense Oligonucleotides

Naturally occurring phosphodiester oligonucleotides are readily available and have low toxicity and activate RNase H. They have displayed activities against some targets *in*

vitro.[27] However, they are degraded rapidly in animals, which limits their use *in vivo*.[28] The key to the future value of oligonucleotide therapeutics resides in the development of modified oligonucleotides. Essentially, all constituents of the oligonucleotide chain: base, sugar, and phosphate backbone, can be modified (Figure 1.3).

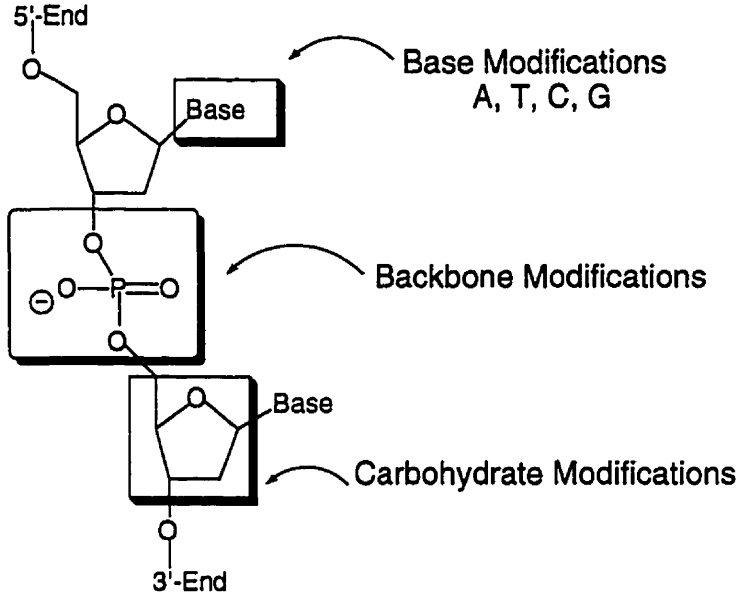


Figure 1.3: Possible Modifications on Natural DNA

Due to the necessity to maintain Watson-Crick hydrogen bonding and base stacking required for oligonucleotide specificity and binding affinity, certain rules have to be followed in carrying out modifications. To target any sequence, modified oligonucleotides require a minimum of four different heteroaromatic structures linked together. A sugar-phosphate moiety provides the linkage between bases in natural nucleic acids. Therefore, base modifications are very limited and only few examples have been reported in the literature with promising antisense applicability.[29] Carbohydrate structural modifications in antisense research have been widely exploited. Several reviews have been published.[30]

The phosphate backbone modifications are the major areas of focus in today's antisense oligonucleotide modifications. They retain the bases of DNA that are essential

for binding and sequence-specificity, and the sugar that allows to orient the base with respect to the backbone axis. The efforts can be divided into the following categories.

1.1.4.1. P-Modified Oligonucleotides

The P-modified oligonucleotides involved the retention of the phosphorus atom and the change of the substitute groups around the phosphorus atom as shown in Figure 1.4.

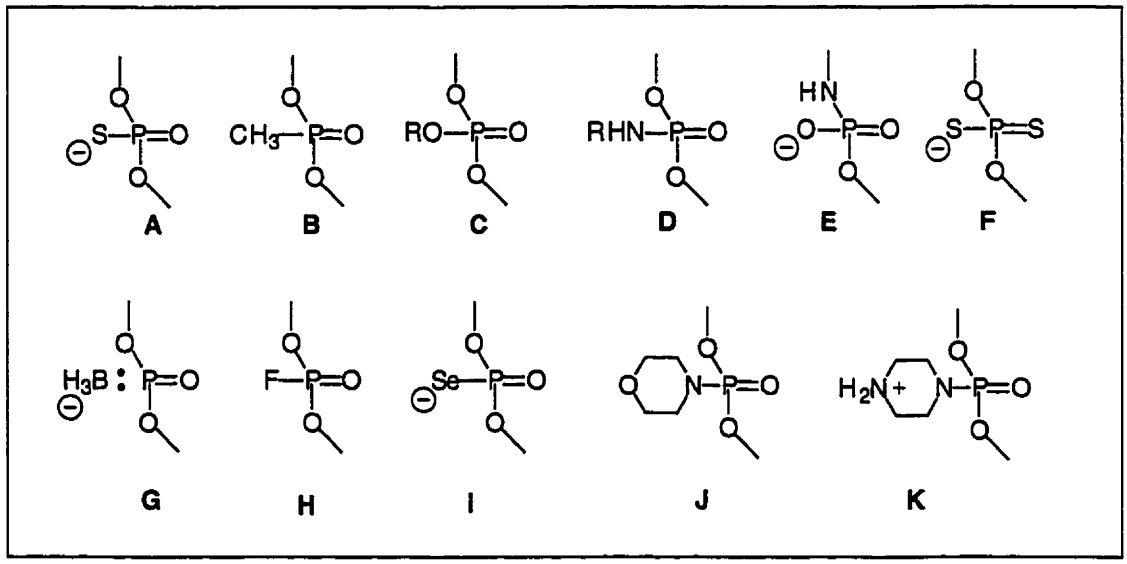


Figure 1.4: P-Modified Oligonucleotides

The discovery of phosphorothioate DNA in 1983 by Eckstein and coworkers marked a significant step forward in the development of antisense research.[31] The phosphorothioate oligonucleotides (**A**)[32] have by far received the most attention as antisense drug candidates. More details about phosphorothioate oligonucleotides will be given in Section 1.2.

The methylphosphonates (**B**)[33] are also received a lot of attentions in antisense research, since they are resistant to nucleases and have good cellular uptake.

The phosphotriesters (**C**),[34] the phosphoramidates (**D, E**),[35] the phosphorodithioates (**F**),[36] the phosphonofluoridates (**H**),[37] phosphoroselenoates (**I**),[38] phosphomorpholidate (**J**), and phosphopiperazidate (**K**)[39] have all been synthesized and tested as possible antisense agents. They all showed better resistance towards exonucleases and endonucleases. Only phosphorodithioates (**F**) and phosphoroselenoates (**I**) showed RNase H activity.

The boranophosphates (**G**)[40] are a new class of antisense oligonucleotides. The recent research development will be described in Section 1.3.

1.1.4.2. Oligonucleotides with Dephosphono Linkages (Linkages without the Phosphorus Atom)

In the last ten years, we have witnessed a steady rise in the number of publications and review articles[41] related to the methods of synthesis and use of nonionic, achiral linkages that replaced the natural phosphate backbone (Figure 1.5).

Replacement of the phosphate backbone of an oligonucleotide has several distinct advantages in terms of its antisense properties: it confers the desired stability towards cellular nucleases; modified oligomers may have increased cellular uptake due to its neutral characteristics; the chirality imposed by the phosphorothioate will be removed; major advantages may be realized in the economics of large scale synthesis in solution.

The synthetic efforts required to create these linkages is often challenging, but they will become more routine with recent advances in synthetic methodologies.

MMI or methylene(methylimino) linkage is a novel backbone modification that has great potential in the oligonucleotide-based antisense therapeutics as a replacement for the natural phosphodiester linkage.[42]

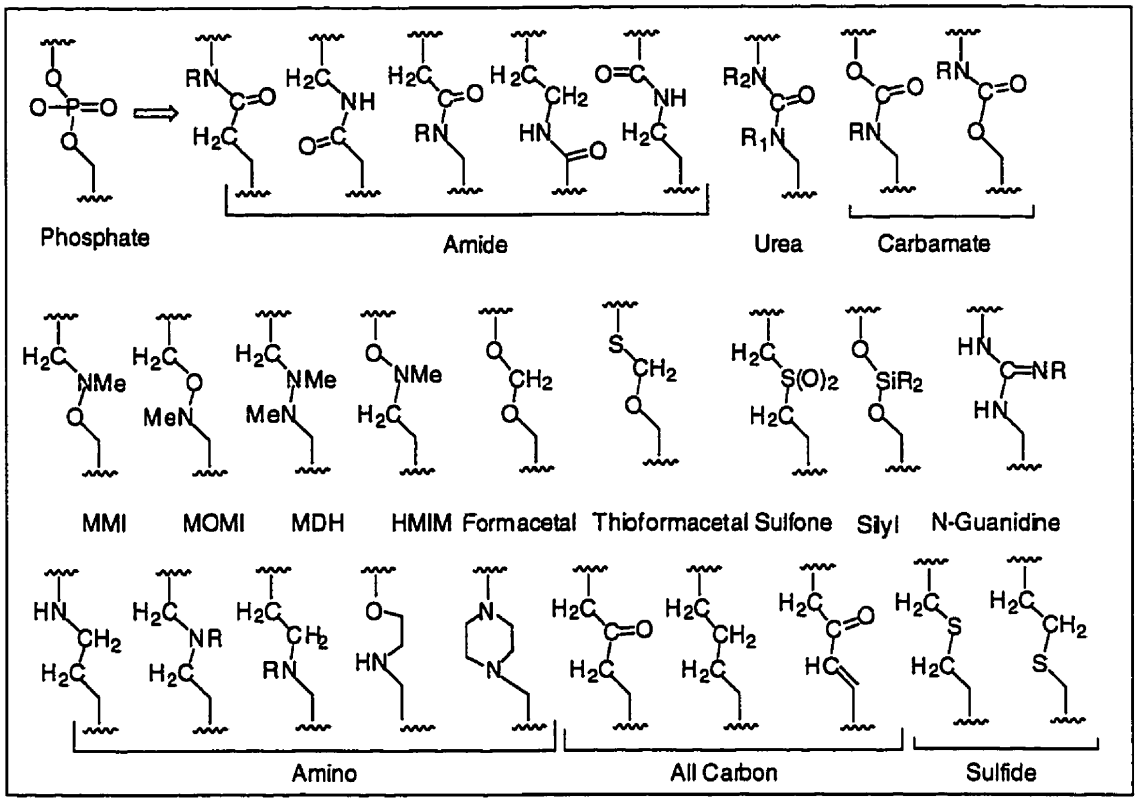


Figure 1.5: Dephosphono Linkages

1.1.4.3. Peptide Nucleic Acid Oligonucleotides

Peptide nucleic acids (PNA) are analogs of oligonucleotides with peptide bonds in the place of the sugar and phosphate backbone.[43] PNA bind strongly and sequence-specifically to both single-stranded DNA and RNA[44] as well as to double-stranded DNA.[45] Furthermore, PNA are both chemically and biologically stable.[46] They can be prepared on large scales at relatively low cost. These properties make PNA attractive leads for the development of gene therapeutics and biomolecular tools.[47] The original PNA backbone consisted of N-(2-aminoethyl)glycine units; the nucleobases are attached to the glycine nitrogen through methylene carbonyl linkers (Figure 1.6).

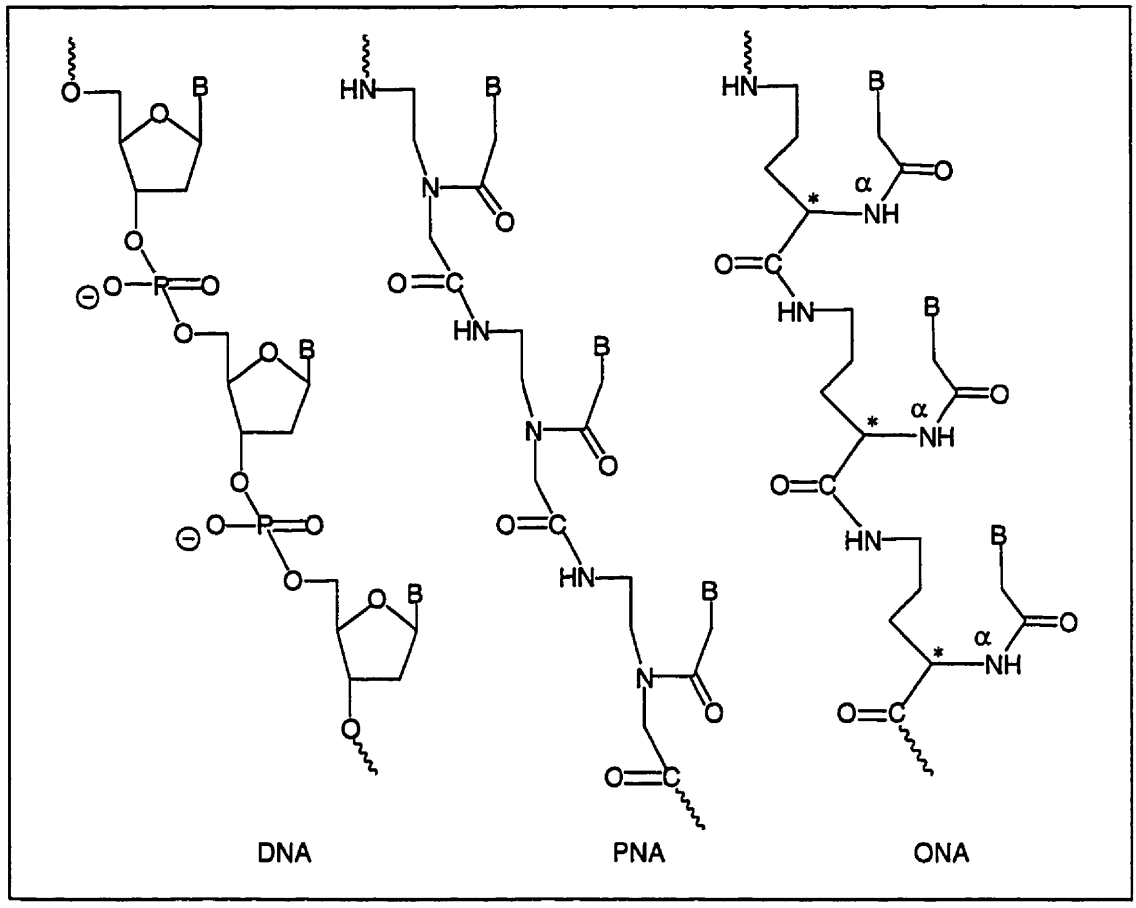


Figure 1.6: The Structures of Phosphodiester Oligonucleotide (DNA), Peptide Nucleic Acid Oligonucleotide (PNA) and Modified PNA Oligonucleotide (ONA)

A number of modified PNA have been prepared. Extension of the PNA backbone by the insertion of methylene units proved to be deleterious to the DNA-mimicking properties.[48] Introduction of chirality into the backbone has also been studied, and the results indicate that chirality arising from methyl groups incorporated at various positions along the backbone hardly affects the hybridization properties.[49]

Later, Lenzi *et al.*[50] prepared a PNA mimic containing aminobutyric acid and glycine as backbone. They demonstrated that this chiral peptidic DNA exhibited self recognition similar to that of DNA/DNA duplexes. Recently, several papers[51] reported a chiral PNA analogue, the backbone of which contains the amino acid ornithine (ONA,

Figure 1.6). The ornithine residue has the requisite six covalent bonds in the monomeric backbone as in PNA. The ONA monomers are linked via amide bonds between the carboxyl and the δ-amino function of the ornithine residue. The nucleobases are attached via a N-acetyl linkage to the α-amino function of ornithine. It was demonstrated that the thymidine decamers can form stable complexes with complementary RNA. Furthermore, it is evident that the chirality of the ONA backbone is one of the factors determining the stability of the complexes.

1.1.4.4. The Second Generation Antisense Oligonucleotides

To enhance the efficacy of phosphorothioates as antisense drugs and to reduce the toxic side-effects, the second generation oligonucleotides have been developed based on the properties of phosphorothioate oligonucleotides. These included chimeric oligonucleotides, hybrid oligonucleotides, self-stabilized oligonucleotides and prodrug oligonucleotides.

(1) Chimeric Oligonucleotides

The rationale for designing chimeric oligonucleotides is based on biophysical, biochemical, and biological properties of various phosphate-modified oligonucleotides, including phosphorothioates.[52] Some of the oligonucleotide analogs, e.g., methylphosphonates, have properties that are different from those of phosphorothioates. Methylphosphonate oligonucleotides are nonionic chemical analogs in which the negatively charged phosphate oxygen is replaced by a neutral methyl group.[53] Methylphosphonate oligonucleotides are highly lipophilic, resistant to nucleases, have weak interaction with plasma proteins, and are taken up by cells by a combination of endocytosis and passive diffusion. The major drawback of these analogs is that they do not activate RNase H. Pharmacokinetic studies show that they are widely distributed in various tissues and are eliminated rapidly in urine.[54] Therefore, in chimeric oligonucleotides, methylphosphonate

internucleotide linkages were incorporated at both the 3' and the 5' ends of the oligonucleotide, whereas the central core was of phosphorothioate composition as shown in Figure 1.7. The methylphosphonate linkages increase the uptake and the stability of the oligonucleotide, whereas the central core enhances the hybridization and activation by RNase H. The overall negative charge of the oligonucleotide as well as binding to plasma proteins is therefore reduced.[55]

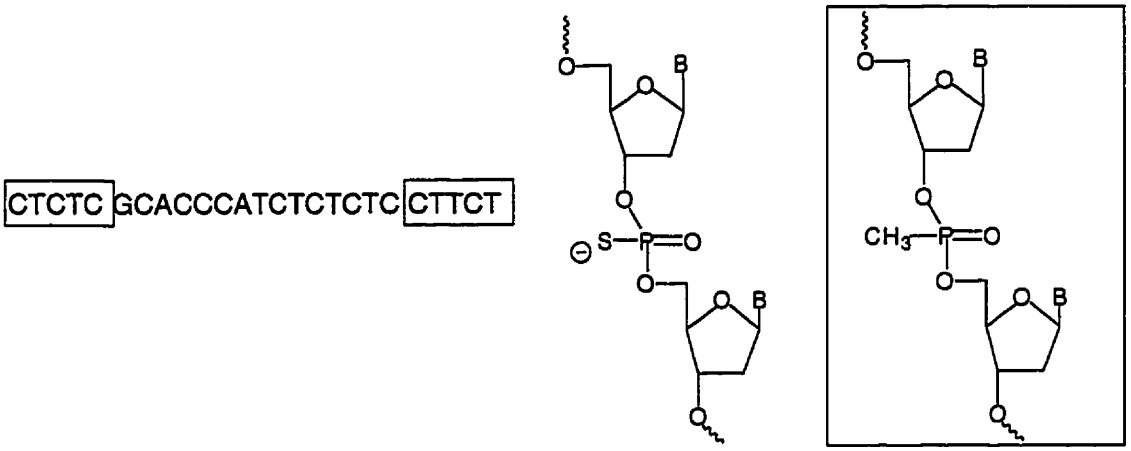


Figure 1.7: Chimeric Oligonucleotide Modifications

(2) Hybrid oligonucleotides

The rationale of designing hybrid oligonucleotides is quite similar to that of the chimeric oligonucleotides. In hybrid oligonucleotides, one or more segments of DNA are combined with one or more segments of modified RNA. For example, the 3' and 5' ends of the oligonucleotide are of 2'-O-methylribonucleoside, whereas the central core is of phosphorothioate composition[56] as shown in Figure 1.8. The mixed 2'-O-methylribonucleotide segment enhances their affinity, selectivity and stability, as a result of the chemical qualities of the RNA-like segments, while still activating the cellular enzyme RNase H. This enzyme destroys the messenger RNA without destroying the antisense compound thereby freeing it to bind with another identical messenger RNA.[57]

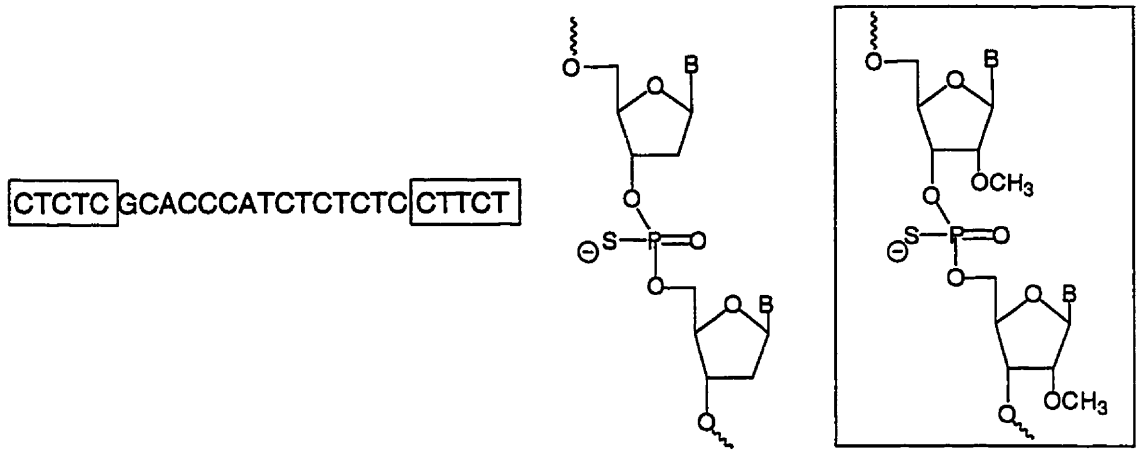


Figure 1.8: Hybrid Oligonucleotide Modification

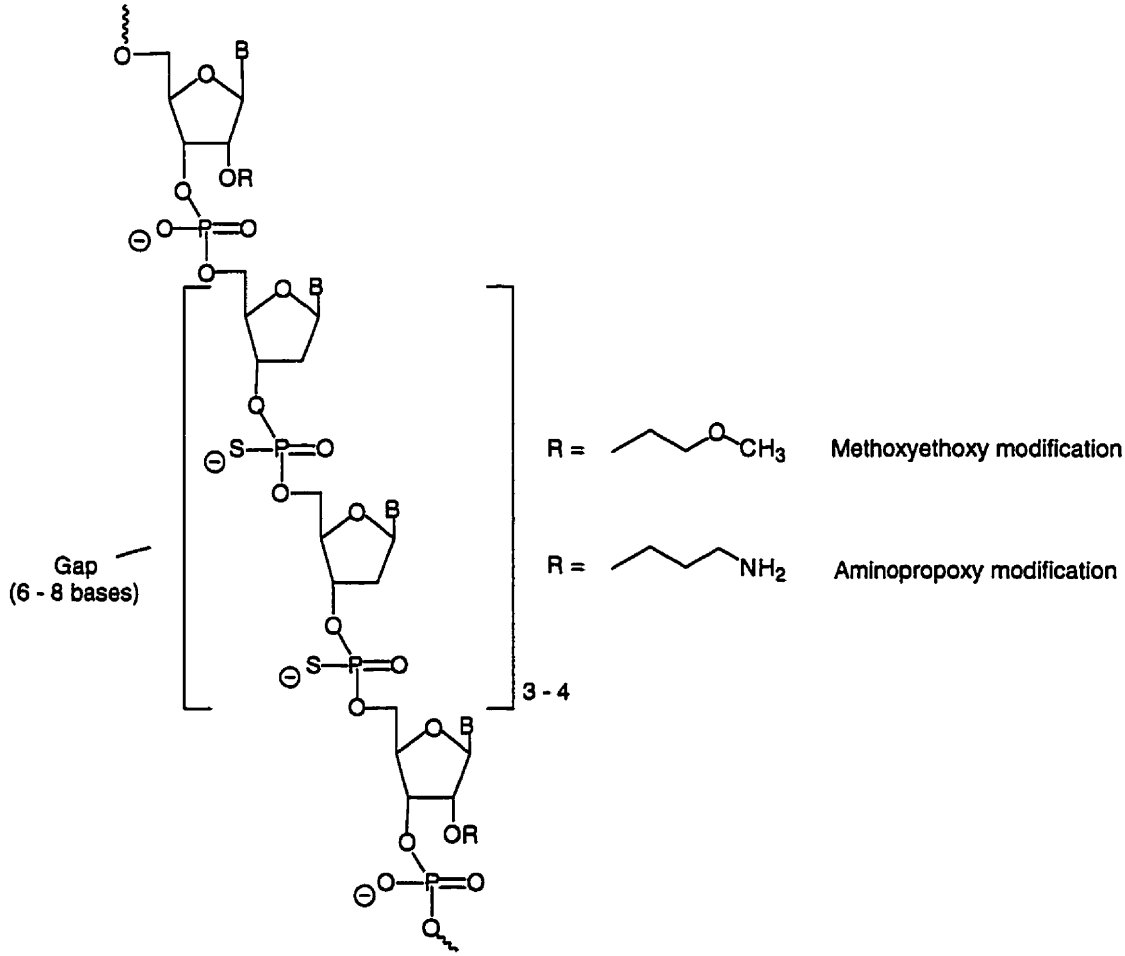


Figure 1.9: End-modified Oligonucleotides Retain Phosphorothioate Core

Recently, other hybrid oligonucleotide modifications have also been reported as shown in Figure 1.9. These modifications involved attaching a 2'-methoxyethoxy or 2-aminopropoxy group at the 2'-position of the ribose ring on nucleotides at each end of the oligonucleotide while leaving phosphorothioate linkages in the middle. They have shown great potential to be good antisense drugs.[58]

(3) Self-stabilized Oligonucleotides

The design of self-stabilized oligonucleotides was based on structural rather than chemical modification (Figure 1.10). Self-stabilized oligonucleotides are of phosphorothioate composition and have one segment that binds by sense/antisense nucleotide bonds to its complementary sequence on the target messenger RNA and another segment that binds by a mechanism other than sense/antisense nucleotide bonds, thus forming a triplex structure. Based on *in vitro* studies, the self-stabilized oligonucleotides have the ability to bind more tightly and specifically with their targets than do normal antisense compounds.[59,60]

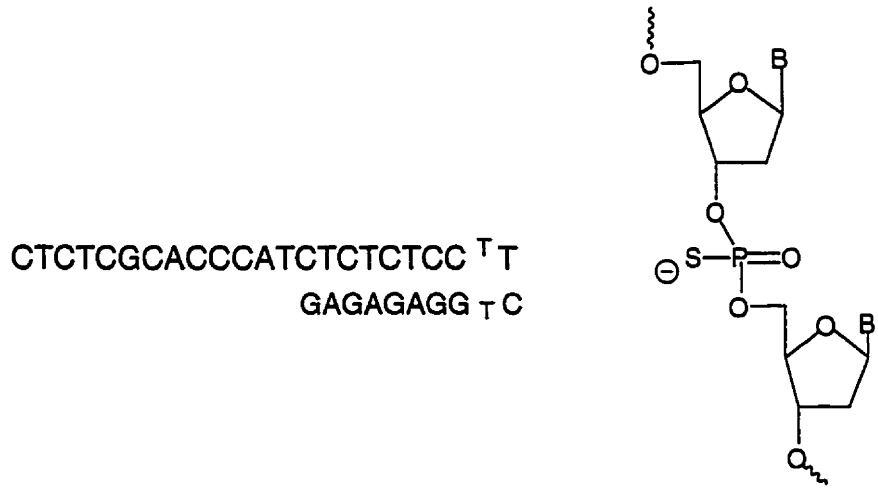


Figure 1.10: Self-stabilized Oligonucleotide Modification

(4) Prodrug Oligonucleotides

The prodrug oligonucleotide in which the backbone is in a triester form can let the body's own esterases hydrolyze the molecule into the parent oligonucleotide after it reaches its cellular target. Because this prodrug is a neutral molecule, it may produce fewer polyanionic side effects and it would pass through cell walls or enter organs by passive diffusion, rather than by the more active process of endocytosis. So far, the biological properties of these prodrugs appear quite promising (Figure 1.11).[58,61]

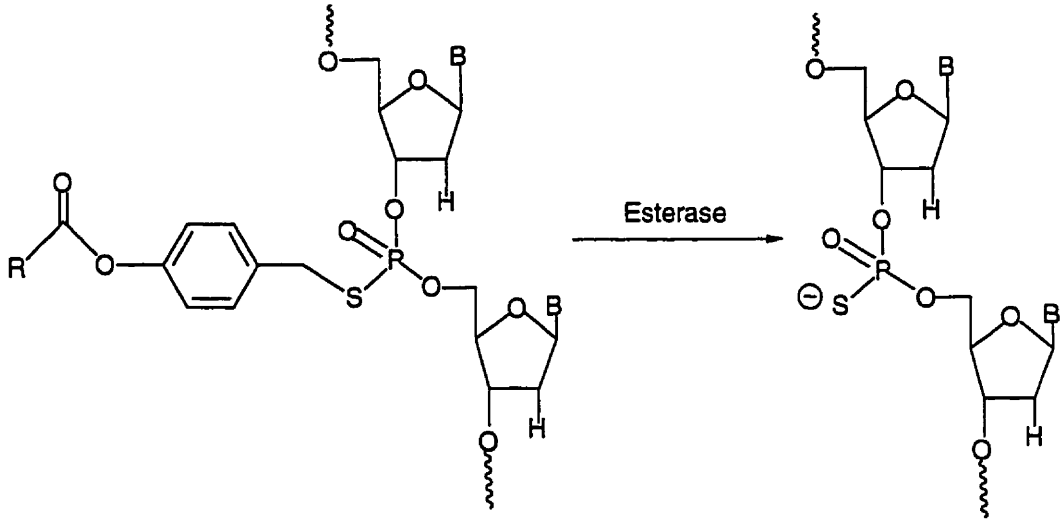


Figure 1.11: Prodrug Oligonucleotide Modification

1.2. Phosphorothioate Oligonucleotides

1.2.1. Phosphorothioate Oligonucleotides As Antisense Drugs

Phosphorothioate oligonucleotides have received the most attention as antisense drug candidates. They have shown resistance to nucleases and having good cellular uptake. Their hybrids with mRNA can activate RNase H, leading to the degradation of the mRNA component and therefore stop the disease-associated protein production. They showed sequence-specificity in most cases, although sometimes they were also associated

with nonsequence-specific effects. The effect of phosphorothioate oligonucleotides as antisense-mediated inhibitors of gene expression is well recognized and their therapeutic potential has been demonstrated in clinical trials against cancer, viral infections and genetic disorders. Presently, phosphorothioate oligonucleotides are the only antisense oligonucleotides that are in clinical trials.[62,63,64]

In oncology, Lynx Therapeutics (Hayward, CA) collaborated with Iversen *et al.*[65,66,67] to develop the first antisense oligonucleotide to enter clinical trial in 1992. Since that time, a number of other phosphorothioates have entered into clinical trials for the treatment of malignant disease (Table 1.1).

Table 1.1: Antisense Oligonucleotides - Cancer Targets in Clinical Development

Gene Target	Oligonucleotide	Sponsor	Backbone-Size	Disease	Reference
p53 exon 10	OL(1)p53	J. Armitage & Lynx*	PS-20 mer	AML-MDS Phase I	65,66,67
c-*myb*	LR-3001	A. Gewirtz & Lynx*	PS-24 mer	CML Phase I	68,69
PKC-α	CGP64128A (ISIS 3521)	ISIS/Ciba	PS-20 mer	Solid tumors-Phase II	70,71,72,73
c-*raf* kinase	CGP69846A (ISIS 5132)	ISIS/Ciba	PS-20 mer	Solid tumors-Phase II	71,72,73,74,75
Ha-ras	ISIS 2503	ISIS		Solid tumors-Phase I	3
bcl-2	G3139	Genta & F. Cotter	PS-18 mer	Lymphoma-Phase I	58,76,77

Abbreviation: PS, phosphorothioate.

*Trials conducted under an investigator IND (Investigation New Drug). Oligonucleotide supplied by Lynx.

In addition to these anticancer programs, antisense oligonucleotides directed against viral or eukaryotic targets for non-cancer indications are in various stages of development (Table 1.2).[78]

Table 1.2: Antisense Oligonucleotides: Viral and Inflammatory Targets in Clinical Development

Gene Target	Oligonucleotide	Sponsor	Backbone-Size	Disease	Reference
HIV gag	GEM91	Hybridon	PS-25 mer	HIV-Phase II	58,73,79
CMV	GEM132	Hybridon	Hybrid PS-20mer*	CMV-Phase I	58,79
HPV	ISIS 2105	ISIS	PS-20 mer	Genial warts-Phase II**	80
HCMV	ISIS 2922 (Fomivirsen)	ISIS	PS-21 mer	HCMV retinitis Phase III completed	73,79,81
HIV	GPs0193	Chugai	PS-26 mer	HIV-Phase I	78
HIV integrase	AR177	Aronex	PO-17 mer***	HIV-Phase I	78
ICAM-1	ISIS 2302	ISIS	PS-20mer	CD, UC, RA, OTR, Psoriasis Phase II	70,71,72,82
c-myc	LR-3280	Lynx	PS-15mer	CAD-Phase II	58
HCMV	ISIS 13312	ISIS	Hybrid PS-Oligos*	Retinitis(AIDs) Phase I/II	3
HIV	GPI-2A	Novopharm Biotech		HIV-Phase I	83

Abbreviations: PS, phosphorothioate. PO, phosphodiester; CD, Crohn's Disease; UC, Ulcerative Colitis; RA, Rheumatoid Arthritis; OTR, Organ Transplant Rejection; CAD, coronary artery disease.

* The second generation antisense drugs; ** Discontinued in 1995; *** Lipid formation

The first antisense drug Isis2922 (Fomivirsen) will be probably on the market in 1998.[3,73,79,81]

1.2.2. The Stereochemistry of Phosphorothioates

The phosphorothioate oligonucleotides currently employed in clinical studies and biological evaluations are obtained as mixtures of 2^n diastereomers, where n is equal to the number of internucleotidic phosphorothioate linkages, since the phosphorothioate oligonucleotides have sulfur in place of oxygen as one of the nonbridging ligands bonded to phosphorus, creating a new center of chirality. This chirality at phosphorus is designed as Rp or Sp (Figure 1.12).

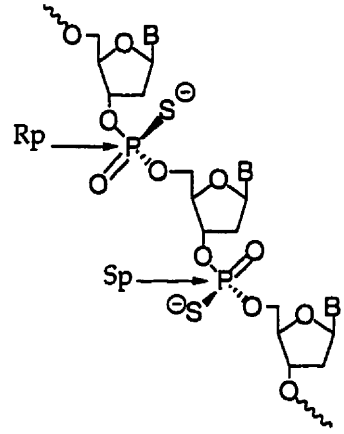


Figure 1.12: The Stereochemistry of Phosphorothioate Linkages

Considering the synthesis of an oligomer of 21 units, the number of possible diastereomers would be 2^{20}, more than one million different molecules.[84] This is clearly undesirable, especially if the mixture is used as a drug.

Molecular recognition in biological systems is often dependent upon the stereochemistry of the molecules involved.[85] Furthermore, two diastereomers are characterized by different physico-chemical properties and consequently different biological properties.[86] For example, phosphorothioate oligonucleotides possessing only (Rp) internucleotidic linkages were found to be resistant to endonuclease P1, whereas the (Sp) oligonucleotides were all cleaved under the same conditions.[87] Contrary to these results,

snake venom phosphodiesterase digested only terminal nucleotides having the (Rp) configuration.[88] Another example is the DNA-RNA complex containing the phosphorothioate oligonucleotides of all-Rp configuration. It was found to be more susceptible to RNase H-dependent degradation compared with hybrids containing either all-Sp counterpart or the so called 'random' mixture of diastereomers.[89] It would be reasonable to think that each diastereomer possessed very particular physico-chemical as well as biological properties and therefore different pharmacological behaviors. We may ask: which diastereomer is responsible to the observed biological response and pharmacological behaviors? This question has initiated lots of interest amongst organic chemists. It is thus very important, from a theoretical and a practical point of view, to develop a methodology for stereoselective synthesis of diastereomerically pure phosphorothioate oligonucleotides.

1.2.3. Synthesis of Phosphorothioate Oligonucleotides (PS-Oligos)

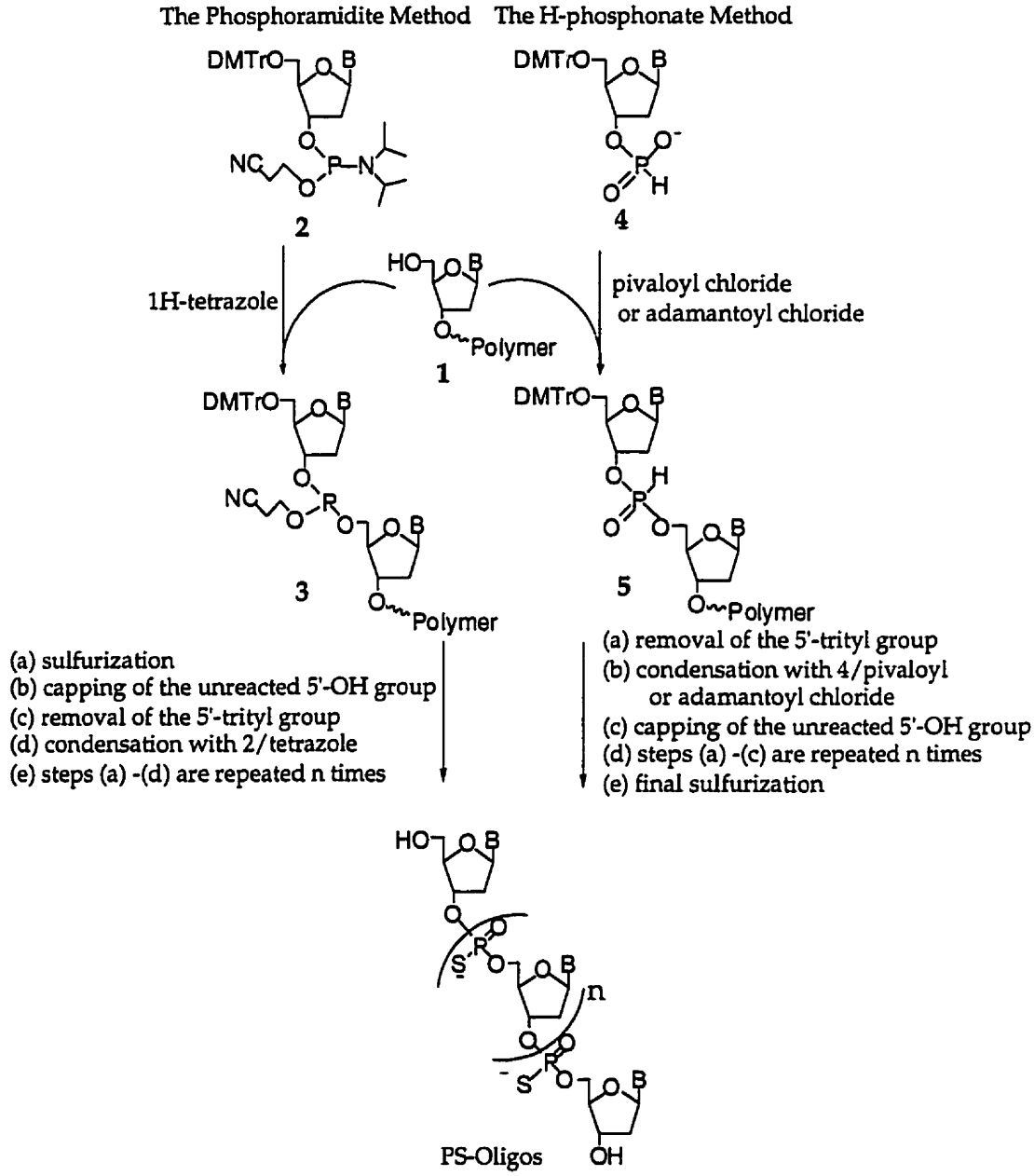
1.2.3.1. General Methods for the Synthesis of PS-Oligos

A factor which makes the PS-Oligos attractive as antisense agents is the fact that they can be prepared in a relatively straightforward manner by modifications to existing automated DNA synthetic techniques. Just like the unmodified oligonucleotides, the PS-Oligos can be routinely synthesized by the automated solid-phase phosphoramidite[90,91] and H-phosphonate method[92] with sulfurization of the phosphite and H-phosphonate intermediates, respectively.

(1) The Phosphoramidite Method

The synthesis of the PS-Oligos via the phosphoramidite approach was first reported by Stec *et al.*[93] Later, Matsukura *et al.*[94] reported an improved version of this method. The method was based on the reaction of the 5'-hydroxy group of a polymer-supported growing DNA chain **1** with a nucleoside cyanoethyl N, N-diisopropyl-phosphoramidite **2**, catalyzed by 1H-tetrazole to provide phosphite triester **3**, followed by (a) sulfurization, (b) capping of the unreacted 5'-

OH group, (c) removal of the 5'-trityl group , (d) condensation with **2**/tetrazole and (e) the steps (a) - (d) are repeated *n* times to provide the final product PS-Oligos (Scheme 1.1).



Scheme 1.1: The General Methods for the Synthesis of PS-Oligos

(2) The H-Phosphonate Method

The advent of practical, automated H-phosphonate chemistry for DNA synthesis, which was first extended by Froehler to phosphorothioate analogues,[92,95] provided for the use of an attractive one-step sulfurization reaction that could be easily and safely performed manually. The starting material of this method involves 5'-O-DMT-nucleoside-3'-O-(H-phosphonate)s **4**, which was activated by trimethylacetyl (pivaloyl) chloride or adamantoyl chloride and reacted with **1** to give O,O-dialkyl-H-phosphonate **5**. This is followed by (a) removal of the 5'-trityl group, (b) condensation with **4** /pivaloyl or adamantoyl chloride and (c) capping of the unreacted 5'-OH group. (d) Steps (a) - (c) are repeated *n* times, followed by (e) final sulfurization to provide the PS-Oligos (Scheme 1.1).

The key feature of the H-phosphonate approach to PS-Oligos is the one-step and very efficient sulfurization. However, the H-phosphonate method has significantly lower coupling efficiency (ca. 95%) than the phosphoramidite method (ca. 99%).

Different sulfurizing reagents have been developed and assessed.[96] Currently, Beaucage's reagent (3H-1,2-benzodithio-3-one 1,1-dioxide) (Figure 1.13) is commonly used on DNA synthesizers as the sulfurizing reagent.[97,98]

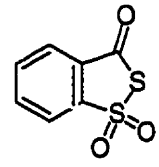


Figure 1.13: Beaucage's Reagent

So far, these two general synthetic methods of PS-Oligos only provided mixtures of diastereomers. As discussed in Section 1.2.2, the control of stereochemistry at the phosphorus center in the synthesis of PS-Oligos appears to be one of the key issues in the development of this class of antisense analogues. However, the methods of stereocontrolled synthesis of P-chiral phosphorothioates are very limited.[84]

1.2.3.2. Stereoselective Syntheses of PS-Oligos

There have several reports of separation of diastereoisomers of phosphorothioate oligonucleotides obtained by means of non-stereocontrolled methods.[99,100] However, the effectiveness of diastereoisomeric oligonucleotide separation depends on the number (n) of modifications within the oligonucleotide chain, in practice, this is limited to n = 4.

(1) Enzymatic Synthesis

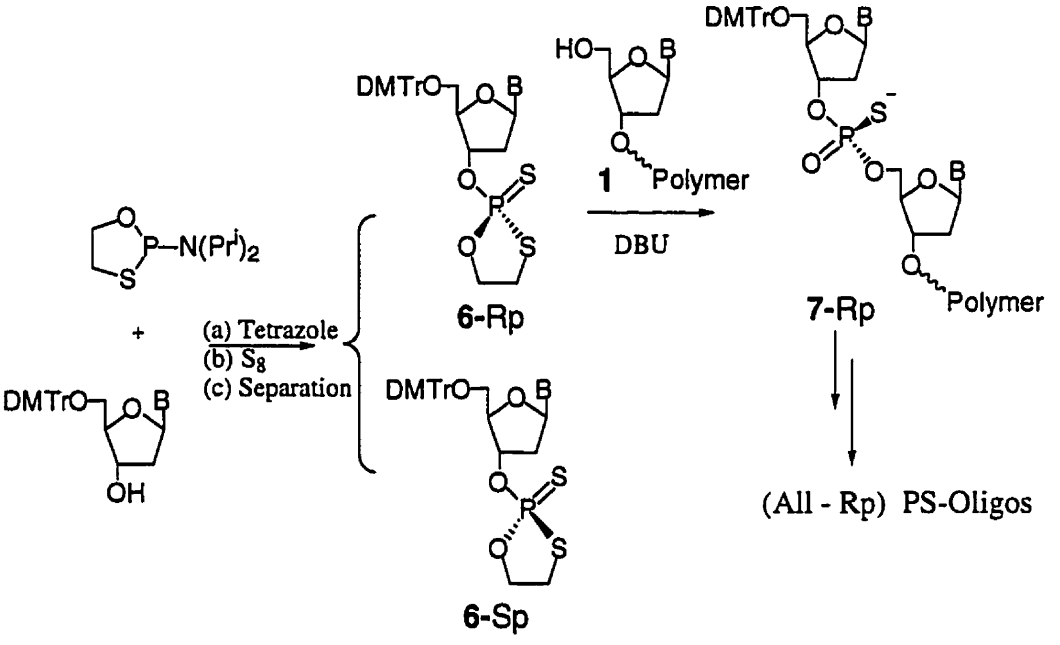
The first enzymatic synthesis of polyribonucleotides containing phosphorothioate linkages was reported by Eckstein and co-workers.[101] They used DNA-dependent RNA polymerase from *E. coli* and synthesized phosphorothioate oligonucleotides having the Rp configuration.[102,103,104] Since then, several polymerases have been found to be able to catalyze the stereospecific formation of phosphorothioate oligonucleotides having consistently the Rp configuration. DNA-dependent DNA polymerases from *E. coli*,[105,106] Phage T4,[107,108] Phage T7,[109] *Micrococcus luteus*,[110] from polynucleotide phosphorylase,[111,112] tRNA nucleotidyl transferase,[113] RNA ligase[114] and 2'-5'-oligoadenylate synthetase[115,116] were all found to be able to catalyze the formation of 3' - 5' or 2' - 5' internucleotidic phosphorothioate linkages in a diastereospecific manner, always leading to a Rp configuration.

The enzymatic synthesis of P-chiral phosphorothioates is limited to short sequences, and the yield is very low. The only rational approach to the synthesis of PS-Oligos having a predetermined sequence of nucleotides and predetermined chirality at each phosphorus atom requires a stereoselective method.

(2) The Oxathiaphospholane Method

So far, the most advanced method for stereocontrolled synthesis of PS-Oligos is Stec's oxathiaphospholane method which was based on nucleophilic substitution at tetracoordinate phosphorus centers.[117,118,119] It has been proved that the reaction of Rp or Sp isomers of 5'-O-dimethoxytrityl-2'-O-deoxyribonucleoside 3'-O-(2-thiono-1,3,2-

oxathiaphospholanes) **6** with 5'-hydroxyl function of support bound nucleoside or growing oligonucleotide chain **1**, performed in the presence of DBU as catalyst , leads stereospecifically to the formation of internucleotide phosphorothioate function 7 in > 95% yield after removal of protective groups and such a process can be applied to the automated synthesis (Scheme 1.2).



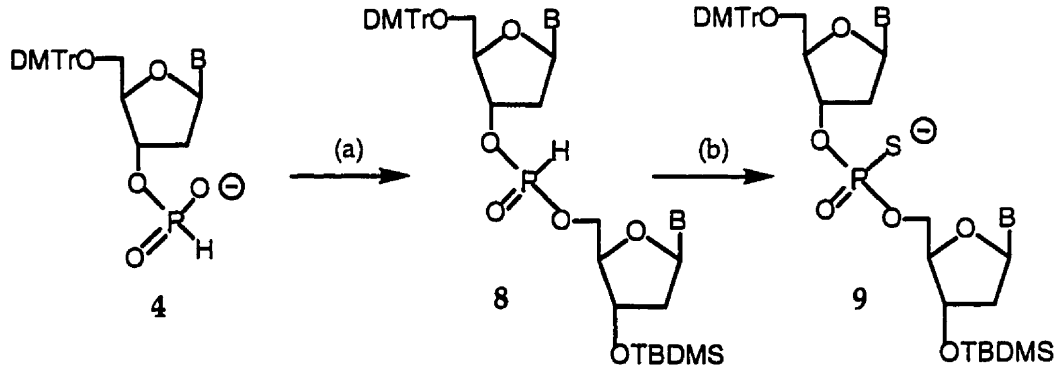
Scheme 1.2: The Oxathiaphospholane Method

However, this method needs chromatographic separation of the oxathiaphospholane diastereomeric mixtures. Therefore, it is not applicable to large scale preparation, and clearly if phosphorothioate oligonucleotides with a defined stereochemistry are to be used in large quantities, a method that allows their synthesis on a large scale still remains to be developed and optimized.

Besides the oxathiaphospholane method, other efforts have been made to develop the methodology for the stereoselective synthesis of phosphorothioate oligonucleotides.

(3) The H-phosphonate Approach

As seen previously, the H-phosphonate approach to the phosphorothioate synthesis is not stereocontrolled. The ratio of diastereomers of di(deoxyribonucleoside)3', 5'-H-phosphonate **5** is known to be close to 1:1.[120,121,122] However, Stawinski *et al.*[123,124] reported that diribonucleoside 3', 5'-H-phosphonate $C_{PH}U$ prepared by their method consisted of a mixture of diastereomers in a ratio of 6:1. Independently, Seela and co-workers have demonstrated that diastereomers of dinucleoside 3', 5'-H-phosphonates **8** can be separated by silica gel chromatography. Furthermore, they proved that sulfurization of the diastereomerically pure dinucleoside H-phosphonates is a stereoretentive process[121] (Scheme 1.3). Therefore, a stereocontrolled synthesis of oligo(nucleoside H-phosphonate)s as convenient precursors to PS-Oligos seems to be a possible method.

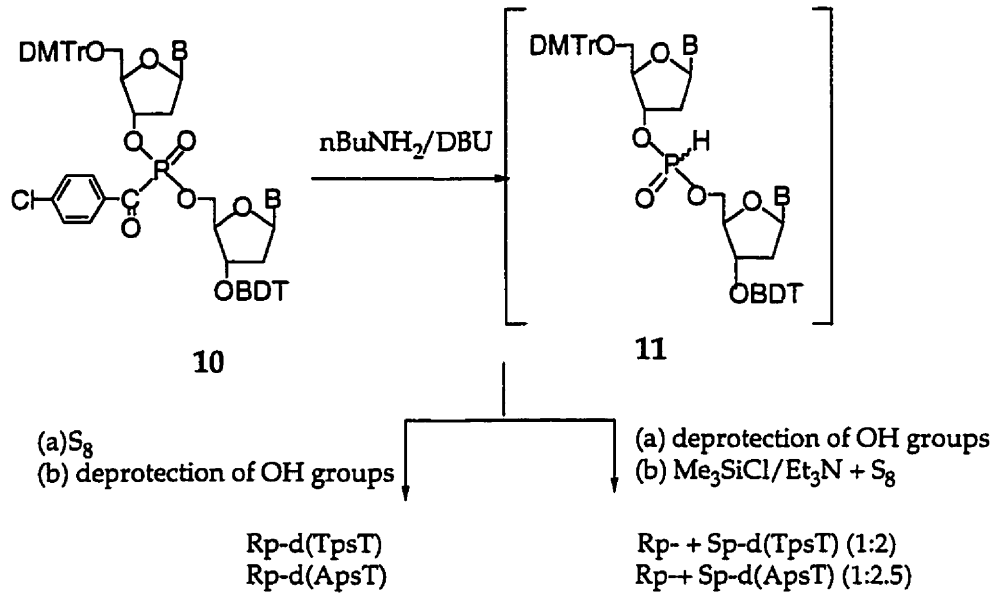


4 8 9

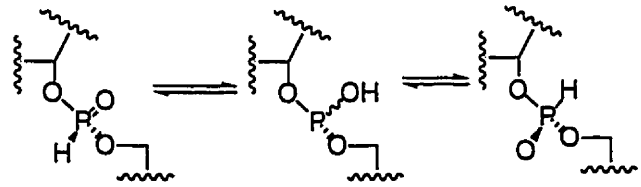
(a). 3'O-TBDMS-nucleoside, t-BuCOCl, Pyridine; (b). S_8 in CS_2/pyridine

Scheme 1.3: The H-Phosphonate Approach (1)

Recently, Hata and co-workers[125] found that treatment of a diastereoisomeric mixture of acylphosphonate **10** with *n*-butylamine, DBU and sulfur led either pure Rp-dinucleoside 3', 5' -phosphorothioates exclusively or a significantly enriched mixture of diastereomers Sp:Rp = 2.5:1, depending on the kind of protective groups and the sequence of deprotection/sulfurization



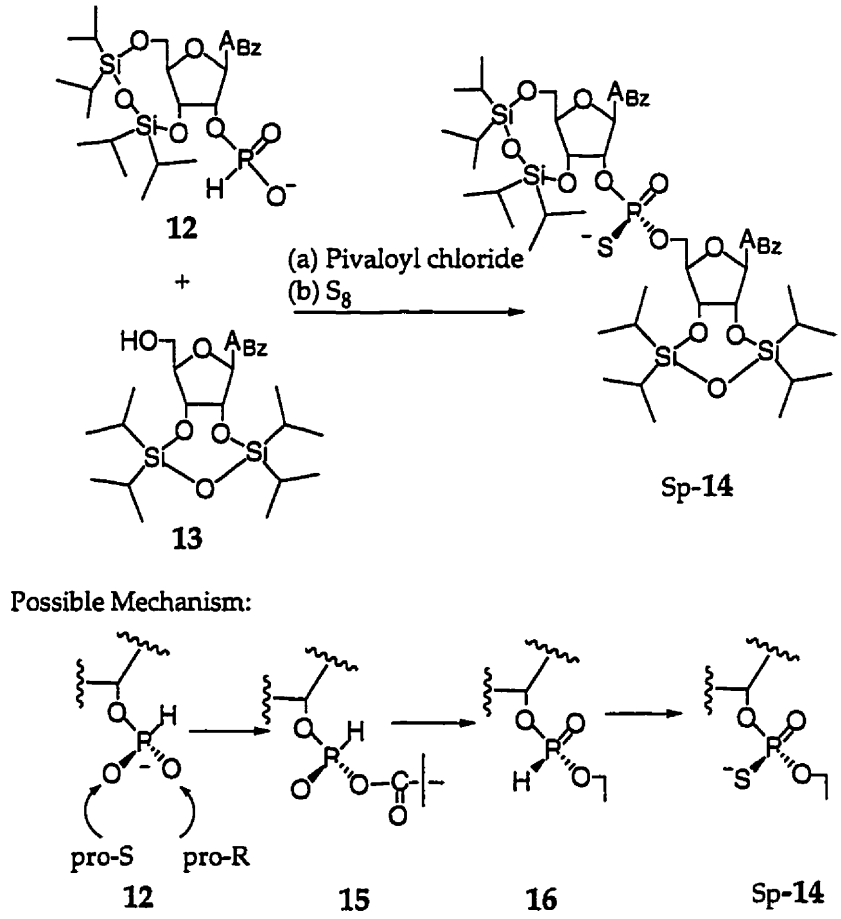
The effect of DBU in shifting the configurational equilibrium of **11**:



Scheme 1.4: The H-phosphonate Approach (2)

steps (Scheme 1.4). As pointed out by the authors in the case of d[TpsT], when a benzoyl or *tert*-butyl dimethylsilyl group was used as the 3'-terminal protecting group instead of 1,3-benzodithiol-2-yl (BDT), the formation of the phosphorothioates was not stereoselective. As an explanation for the observed phenomenon, the authors suggested a mechanism involving DBU catalyzed isomerization of dinucleoside H-phosphonate **11** formed as an intermediate from suitable aroylphosphonates at the first step of aroylphosphonate phosphorothioate transformation. DBU influenced the configuration equilibration of the resulting H-phosphonates **11**. A nucleophilic attack of DBU molecule on the phosphorus atom of H-phosphonate would result in a pentavalent intermediate which would be isomerized by

pseudorotation, DBU elimination would then lead to the thermodynamically more stable H-phosphonates. These ligands may be in equilibrium depending strongly upon the character of the ligands connected to the phosphorus atom. A similar intermediate was isolated by Merckling and Rüedi.[126]



Scheme 1.5: The H-phosphonate Approach (3)

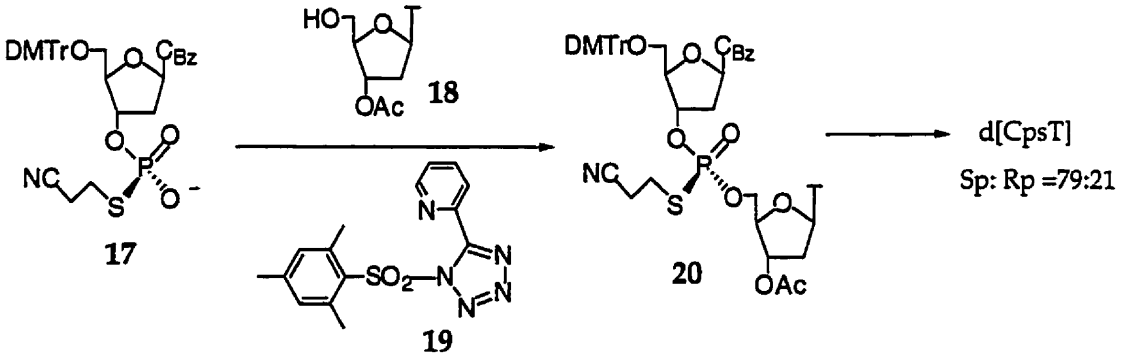
Another example using H-phosphonate chemistry for the stereocontrolled synthesis of phosphorothioates was described by Battistini *et al.*[127,128] Condensation of N^6-benzoyl-3', 5'-O,O-tetraisopropyldisiloxane adenosine 2'-O-(H-phosphonate) **12** with **13** in the presence of pivaloyl chloride provided exclusively Sp-diadenosine 2',5'-phosphorothioate **14** after sulfurization (Scheme 1.5). The interpretation of this result was the presence of sterically

demanding protective groups at the nucleoside components. Presumably, for steric reasons, acylation of anionic **12** occured exclusively at the pro-R oxygen atom, and anhydride **15** reacted stereospecifically with **13** leading to R_p-2',5'-$A_{PH}A$ **16**. Subsequent stereoretentive sulfurization gave S_p-**14**.

In both H-phosphonate approaches presented above, the synthesis of phosphorothioate dimers was highly diastereoselective. However, it did not seem to be applicable to the synthesis of longer oligomers and no further studies about these approaches were reported.

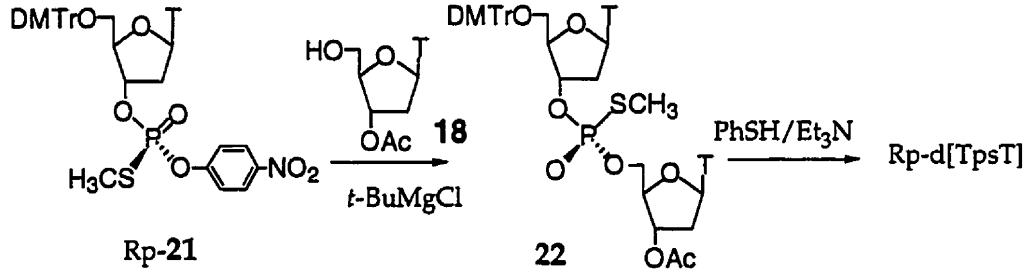
(4) Other Possible Approaches

One approach to the stereocontrolled synthesis of phosphorothioate, based on the chemistry of tetracoordinated phosphorus atom, was reported by Crosstick and Williams.[129] They demonstrated that the reaction of **17** with 3'-O-acetylthymidine **18** in the presence of (2,4,6-trimethylbenzenesulfonyl)-5-(pyridin-2-yl)tetrazole **19** as the activating agent led to the formation of the corresponding dinucleoside **20**, which after decyanoethylation consists of the diastereomeric mixture of d(CpsT) with a distinctive preponderance of the Sp diastereomer. In the best case the ratio of diastereomers Sp:Rp was 79:21 (Scheme 1.6). Such enrichment, although not satisfactory in terms of a stereocontrolled synthesis of PS-Oligos, was worth deeper consideration and perhaps more extensive study.



Scheme 1.6: The Stereoselective Synthesis of d[CpsT]

Another promising and straightforward approach to the stereoselective synthesis of phosphorothioate was stereoselective nucleophilic substitution at tetracoordinate phosphorus centers. Lesnikowski *et al.*[130,131] reported that the separated diastereomers of **21** reacted with 3'-O-acetyl nucleosides **18** in the presence of *t-BuMg*Cl to give dinucleoside 3', 5'- (S-methyl)phosphorothioates **22** (Scheme 1.7). Cleavage of the CH_3-S bond (phosphorothioate deprotection) was achieved in a later step with $PhSH/Et_3N$. The coupling occured with inversion of configuration at phosphorus with a stereospecificity of > 95% and furnished d[TpsT] in about 70% yield.

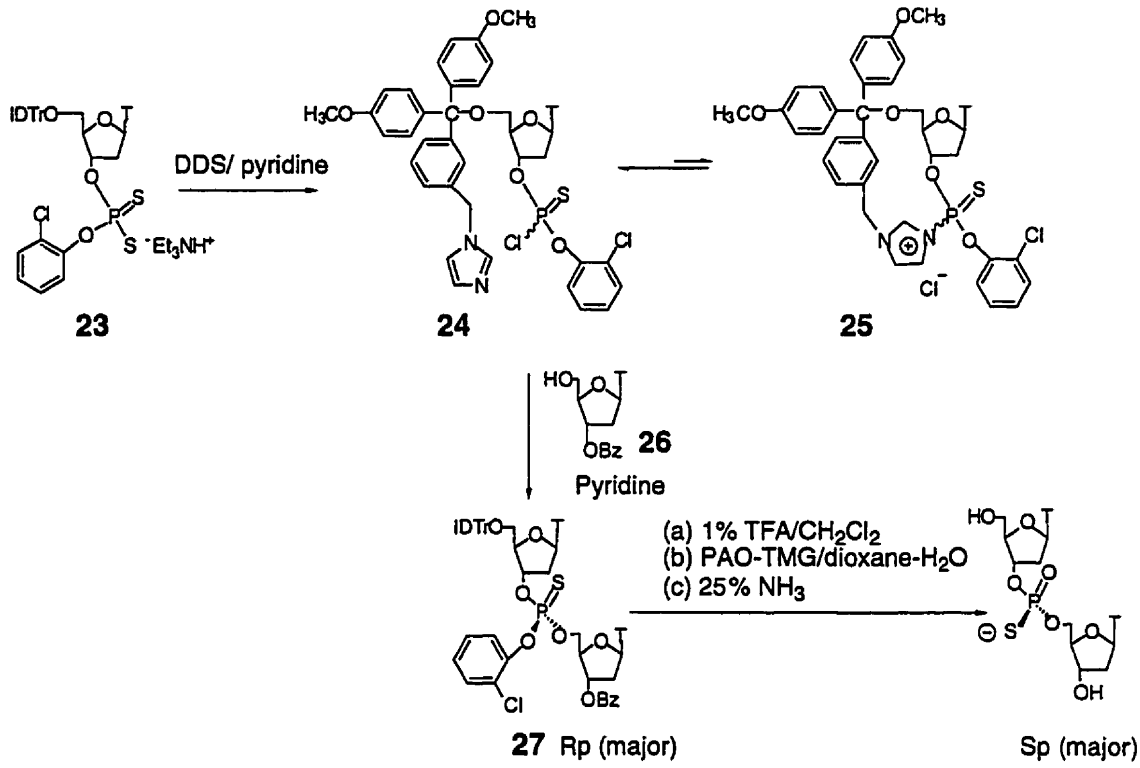


Scheme 1.7: Stereospecific Synthesis of d[TpsT]

Although this method cannot be used in solid-phase synthesis, Lesnikowski *et al.*[132] were able to demonstrate that preparation of stereochemically pure trimers d[TpsTpsT] and tetramers d[TpsTpsTpsT] was feasible if protective group such as 4-chlorobenzyl was introduced at sulfur. This strategy was also applied to the synthesis of (Rp,Rp)- and (Sp, Sp)-UpsUpsU.[133]

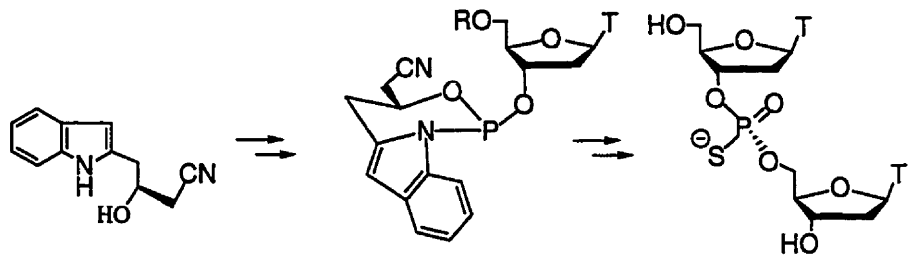
Very recently, Wada *et al.*[134] reported a phosphotriester approach to the stereoselective synthesis of dithymidine phosphorothioates by use of 3-(imidazol-1-ylmethyl)-4',4''-dimethoxytrityl (IDTr) as a 5'-hydroxyl protecting group (Scheme 1.8). The IDTr-catalyzed condensation between **23** and **26** in pyridine yielded the dimer **27**, through an intermediate **24**. After removing protecting groups, Sp dithymidine phosphorothioate was obtained as the major

isomer. The method required neither diastereomerically pure starting materials nor reactive intermediates.



Scheme 1.8: The Phosphotriester Approach

In our research group, besides the phosphoramidite approach to the stereoselective synthesis of chiral PS-Oligos which will be presented in this thesis, Wang[135] demonstrated that the use of **28**-(S) or **28**-(R) as chiral auxiliary could lead to the stereoselective synthesis of dinucleotide phosphorothioates with a de of > 98%, through an indole-oxazaphospholane intermediate **29** (Scheme 1.9).



28-(S) **29** Rp

Scheme 1.9: The Indol-oxazaphosphorine Approach

1.3. Boranophosphate Oligonucleotides

1.3.1. Boranophosphate Oligonucleotides As Potential Antisense Reagents

Boranophosphate oligonucleotides are new members of modified oligonucleotides with a boron-substituted phosphate group (Figure 1.14). The borane moiety ($-BH_3$) in the boranophosphate is isoelectronic with oxygen in naturally occurring phosphodiesters and isoelectonic and isosteric with the $-CH_3$ group in methylphosphonates. The boronated oligonucleotides carry the same negative charge as normal phosphodiesters and phosphorothioates and, like them, are soluble in aqueous solutions. However, they are more hydrophobic than normal phosphates due to the presence of a borane group that should increase their membrane permeability. Furthermore, since the $-BH_3$ group in boranophosphates is isoelectronic as well as isosteric with the $-CH_3$ group, it might be expected that boronated oligonucleotides would also exhibit some desirable properties of the methylphosphonate nucleotides, which form stable hybrids with DNA and may enter cells through passive transport mechanisms.[40,136]

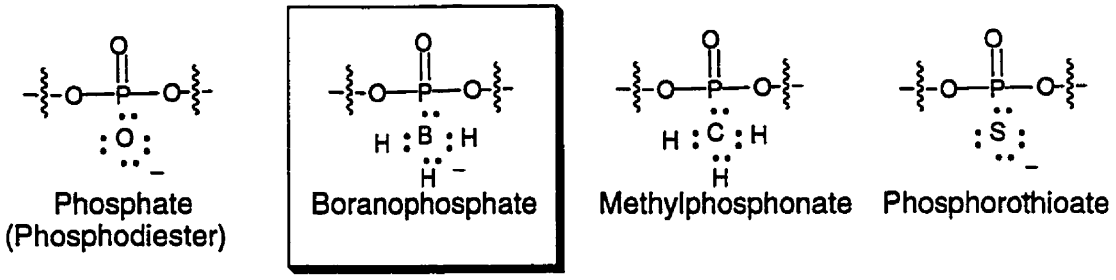
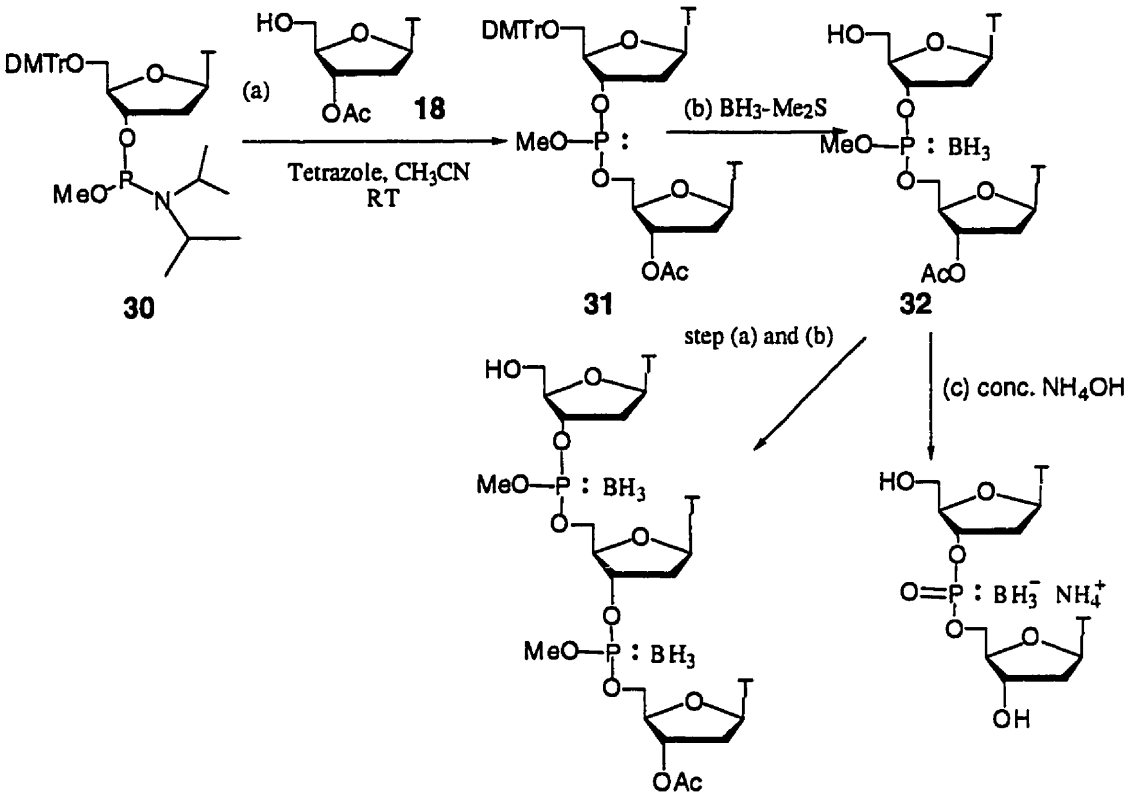


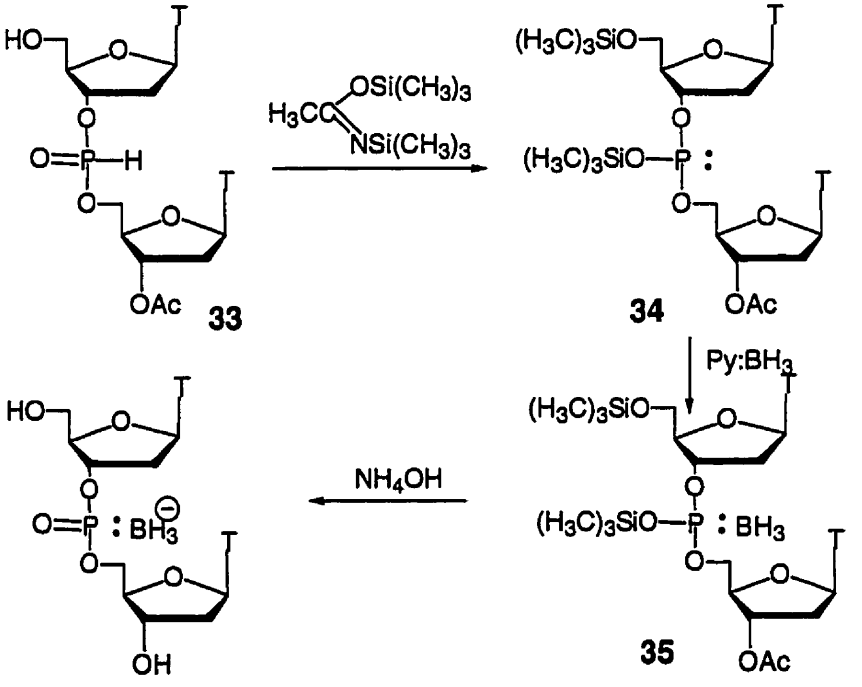
Figure 1.14: Internucleotide Linkage Modifications

The negligible conformational changes of an oligonucleotide chain induced by the boranophosphate group and the presence of unmodified nucleoside units provide an opportunity to explore the use of boranophosphate oligonucleotides as antisense and antigene agents.[137] Moreover, boranophosphate oligonucleotides are remarkably stable at physiological conditions in spite of the presence of boron-hydrogen bonds.[138,139] Additionally, substitution of the phosphate groups in oligonucleotides by boranophosphate leads to significant increase in resistance towards various endo- and exonucleases that is important for successful antisense and antigene applications,[140,141] in particular since their hybrid with mRNA may be substrates for RNase H.[142,143]

The boranophosphate linkage was first synthesized in dinucleotides in 1990 by Sood, Shaw and Spielvogel[39] (Scheme 1.10), using the phosphoramidite approach. The triester phosphite group formed during the coupling reaction easily reacted with different borane complexes giving the desired boranophosphate. However, this methodology required boronation in each elongation cycle and therefore a high yield of this reaction was very critical for successful synthesis of long oligonucleotide chains. Therefore, Shaw's group[144] developed a H-phosphonate approach (Scheme 1.11) for the synthesis of boranophosphates. This approach avoided multiple boronation steps and one boronation reaction for all H-phosphonate groups can be performed simultaneously after the complete chain elongation of an oligonucleotide, using mild conditions without base damage and producing the desired boranophosphate oligonucleotides in high yield.

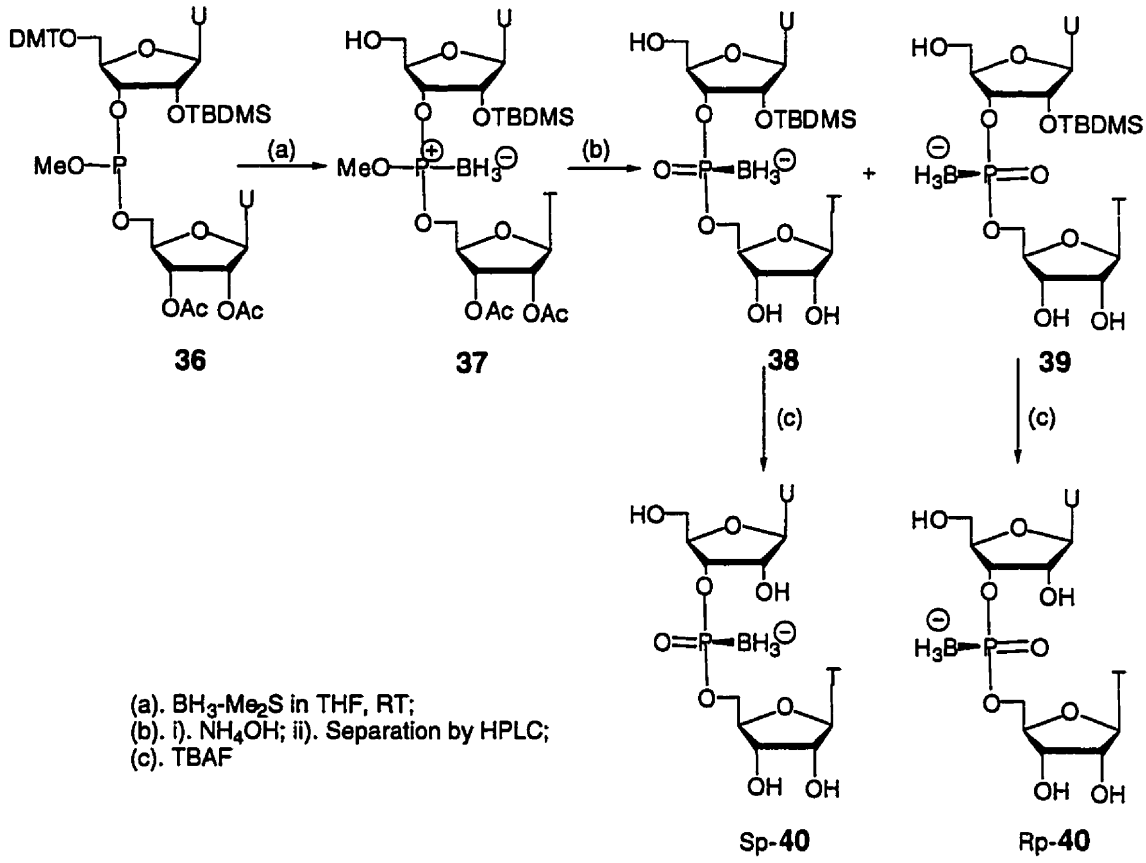


Scheme 1.10: The Synthesis of Di- and Trithymidine Boranophosphates



Scheme 1.11: The H-phosphonate Approach to the Synthesis of Boranophosphates

In 1995, Chen's group[145] reported a synthesis of diuridine 3',5'-boranophosphate (Scheme 1.12). Its diasteromers were separated by reversed-phase HPLC on a C18 column and their configuration was tentatively assigned based on the snake venom phosphodiesterase hydrolysis.



Scheme 1.12: The Synthesis of Diuridine 3',5'-boranophosphates

In 1997, Shaw's group[136] also reported a separation of two diastereomers of dithymidine boranophosphates using reverse phase HPLC (Figure 1.15). A preliminary assignment of the configuration at phosphorus was made on the basis of enzyme selectivity and NOE difference experiments. The Sp and Rp stereochemistry was assigned to the faster eluting isomer d(T_p^BT)-1 and the slower eluting isomer d(T_p^BT)-2, respectively.

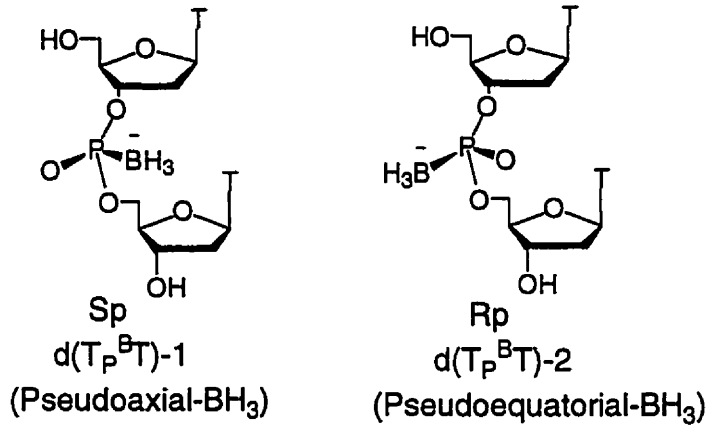
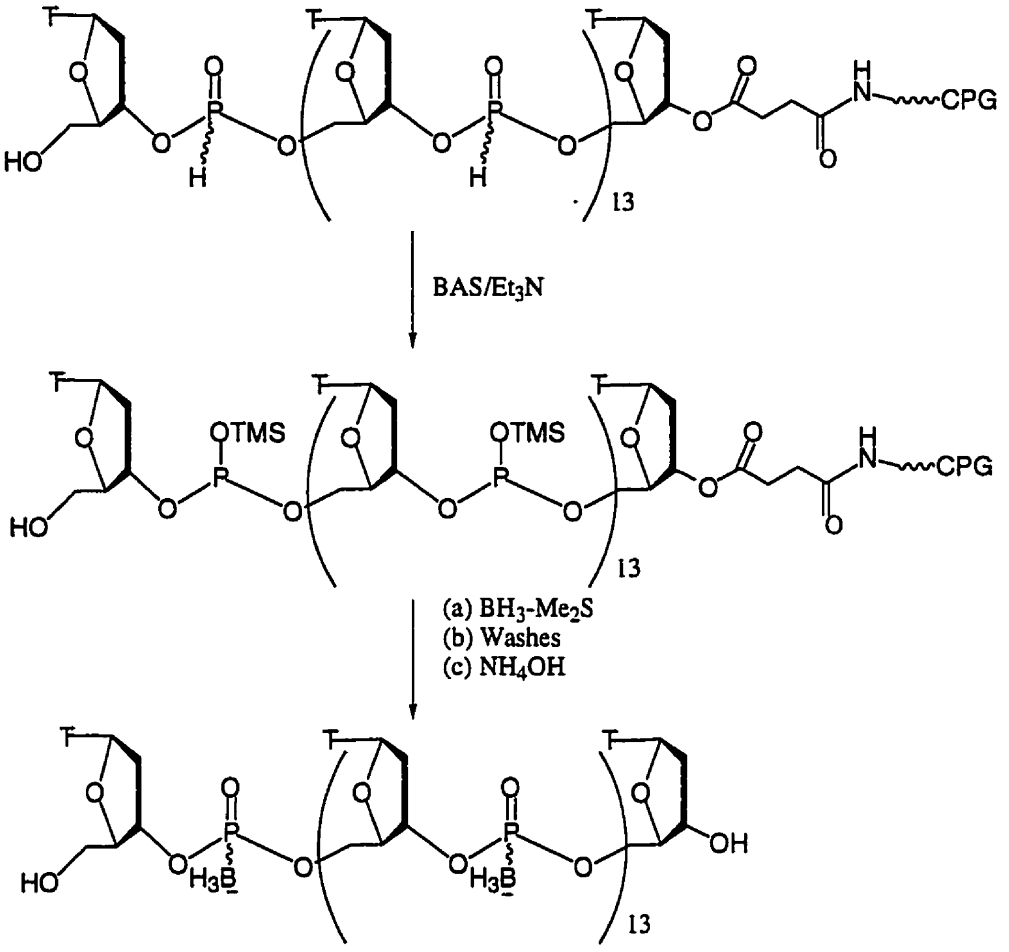


Figure 1.15: Two Diastereomers of Dithymidine Boranophosphates

In 1995, a 14-mer oligodeoxynucleotide (ODN) containing a single Sp boranophosphate linkage was synthesized enzymatically. This ODN bound to a complementary DNA molecule with slightly poorer binding affinity relative to an unmodified control. This study indicates that singly modified backbone-boronated DNA might be a good analog of unmodified DNA.[140]

In 1997, Matteucci's group[146] reported the chemical synthesis of a T_{15} ODN fully substituted with a diastereomeric mixture of boranophosphate linkage (Scheme 1.13). They found that the binding affinity to the diastereomeric mixture of the boranophosphate-linked oligothymidine ODN with complementary RNA and DNA was much poorer than the native phosphodiester ODN control.

Consequently, diastereomeric mixtures of boranophosphate are unlikely to be useful replacements for phosphate diesters in antisense research. The uses of the diastereomerically pure boranophosphates which are attainable from enzymatic methods and potentially from stereocontrolled chemical synthesis, remain an open question.



Scheme 1.13: Chemical Synthesis of a T15 ODN Fully Substituted with Boranophosphate

Recently, Shaw's group[144] reported a synthesis of all-stereoregular boranophosphate oligonucleotides by enzymatic template extension reactions using nucleoside α-boranotriphosphates ([α-P-BH_3]-dNTPs), which were good substrates for a number of polymerases.[138,147,148] However, only relatively small amounts of oligonucleotides can be synthesized by this enzymatic approach. There is still a need to find a chemical method for the synthesis of chiral boranophosphate oligonucleotides.

1.3.2. Boranophosphate Oligonucleotides as Boron Neutron Capture Therapy (BNCT)

Boranophosphate oligonucleotides are of special interest due to the property of non-radioactive boron-10 nuclei to absorb low energy neutrons.[136,149] Boranophosphate oligonucleotides may therefore offer a unique advantage over other modified oligonucleotides as boron neutron capture therapy (BNCT) agents.

$$^{10}B + {}^{1}n \rightarrow (^{11}B) \rightarrow {}^{7}Li + {}^{4}He + 2.4\ MeV^{150}$$

When the stable isotope boron-10 is irradiated with thermal neutron, an alpha (α) particle (helium) and lithium-7 nuclei are released through a nuclear reaction producing about 100 million times more energy than that was initially used. The generated radiation destroys the target tumor cells without damaging normal tissues in the process.[151] This combined modality, originally proposed by Locher in 1936,[152] is known as BNCT.

With boronated oligonucleotides acting as antisense agents, it may be possible to accumulate the boronated oligomers selectively in abnormal cells, such as those infected by a virus, and then to destroy the targeted cells by using boron neutron capture therapy.

The design and synthesis of boron compounds that will specifically localize in the nuclei of tumor cells and bind to DNA would be very desirable and may be quite feasible.[153,154] A number of types of modified nucleic acids are known to be incorporated in the cell nucleus. A boron-containing thymidine analog, 5-dihydroxy-boryl-2'-deoxyuridine, not only incorporates into the cell nucleus, but also significantly radio-sensitizes the cell.[155] Thus it is reasonable to expect that a similar effect may be observed with boron-containing oligonucleotides. Another important consideration for BNCT efficacy is the amount of boron present in the cell. Various calculations estimate that for BNCT to be effective, the amount of boron in the cell should be approx. 5-20 μg/g of tissue if it was distributed uniformly over the cell. If, however, the boron was targeted[156,157] to the RNA or DNA of the cell (e.g., if boronated oligonucleotides were able

be gained by attaching various tumor-seeking groups, such as porphyrins[158] or other hydrophobic groups[158,159] to the 5'- or 3'-ends.

Besides being potential reagents for BNCT and antisense oligonucleotide therapeutics (AOT), the use of boranophosphate oligonucleotides as molecular probes is another important future practical application.[160]

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Chapter 2

The Stereoselective Synthesis of Phosphorothioates

2.1. Introduction

The effect of oligonucleotide phosphorothioates (PS-Oligos) as antisense inhibitors of gene expression is well recognized,[1] and their therapeutic potential has been demonstrated in clinical trials against AIDS and cancer.[2] The PS-Oligos currently employed in clinical studies and biological evaluations are obtained as mixtures of 2^n diastereomers, where *n* is equal to the number of internucleotidic phosphorothioate linkages. To date only one generally applicable stereoselective synthesis methodology of PS-Oligos has been described by Stec *et al.*[3] It unfortunately can not be scaled up due to a difficult chromatographic separation of the required chiral precursors. Therefore, there still exists a need to develop practical stereoselective syntheses of PS-Oligos.

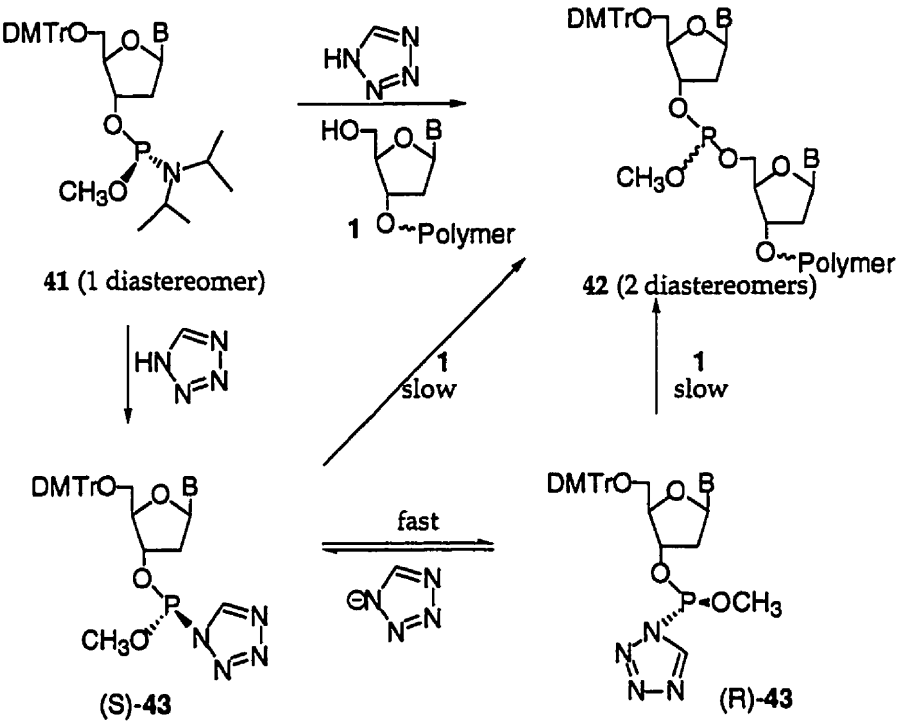
2.2. The Rational and Objectives of the Research

2.2.1. The Phosphoramidite Approach

The phosphoramidite method to the non-stereocontrolled synthesis of PS-Oligos was well established and very efficient. It seemed therefore very important to modify it to prepare chiral PS-Oligos, rather than developing a new technology.

The classical phosphoramidite method leads to PS-Oligos with no control of stereochemistry on the phosphorus atom because the substitution is done on a diastereomeric mixture of phosphoramidite. Therefore, it was first thought that the diastereomerically pure phosphoramidite **41** would lead to a phosphite triester **42** with a well defined sense of chirality on the phosphorus atom, followed by stereoretentive sulfurization to give the P-chiral phosphorothioate. However, a diastereomer mixture of phosphite triester **42** was obtained because of the P-epimerization at the phosphorus atom[4] (Scheme 2.1). Detailed mechanistic studies confirmed this mechanism.[5,6,7] Besides protonation, activation of **41** by tetrazole also involved the substitution of the diisopropylamino group by tetrazole. Intermediate

phosphorotetrazolidites **43** undergo fast P-epimerization in the presence of an excess of tetrazole, most probably by rapid exchange of substituents. Eventually, the phosphite triester **42** was obtained as a mixture of two diastereomers and the stereoselectivity was completely lost, constituting the reason why the phosphoramidite approach was abandoned early in the attempts to synthesize diastereomerically pure PS-Oligos.



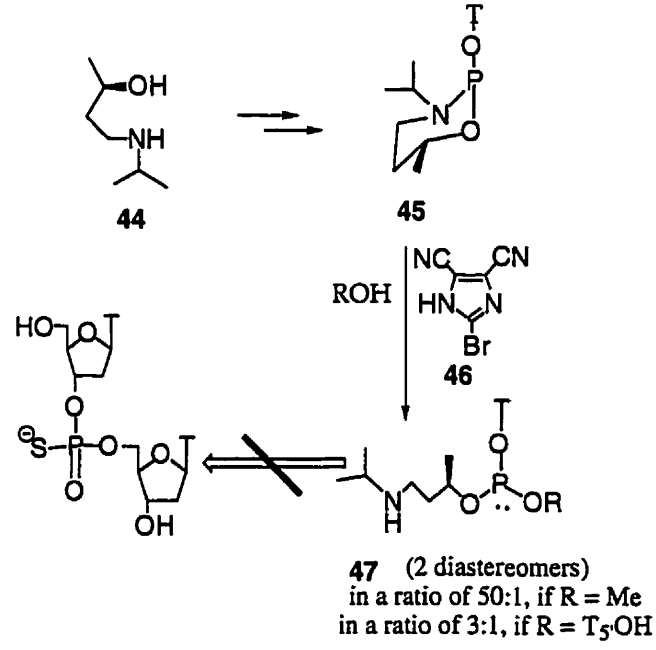
Scheme 2.1: The Mechanism of P-epimerization

2.2.2. Towards the Stereoselective Synthesis of Phosphorothioates by Using the Phosphoramidite Approach

In order to adapt the phosphoramidite approach to a stereocontrolled synthesis of phosphite triesters, and therefore phosphorothioates, two essential requirements had to be met. The first one was the control of stereochemistry at the phosphorus center in the phosphoramidite, i.e. to synthesize a diastereomerically pure and stable phosphoramidite to

avoid the separation of diastereomers. The second one was the control of stereochemistry during the coupling step to avoid P-epimerization.

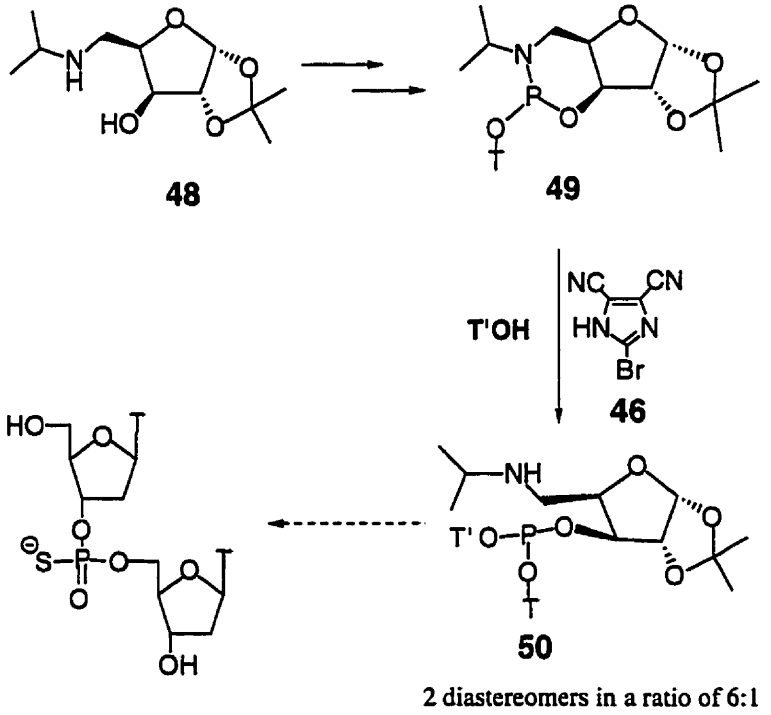
In our research group, Xin[8] had synthesized the six-membered cyclic phosphoramidite **45** by using a chiral γ-aminoalcohol **44**. The two diastereomers of the phosphoramidite were obtained in a ratio of 20:1. Furthermore, using 2-bromo-4,5-dicyanoimidazole **46** as catalyst in the coupling reaction gave a good stereoselectivity. However, this work had some limitations: (i) when a small alcohol, such as methanol, was used in the coupling reaction, the diastereomeric ratio was 50:1, but when a nucleoside was used in the coupling reaction, the stereoselectivity decreased to 3:1; and (ii) the chiral auxiliary could not be removed at the end of synthesis (Scheme 2.2).



Scheme 2.2: Towards the Stereoselective Synthesis of Phosphorothioates by Using the Phosphoramidite Approach (1)

2.2.3. The Objectives of the Research

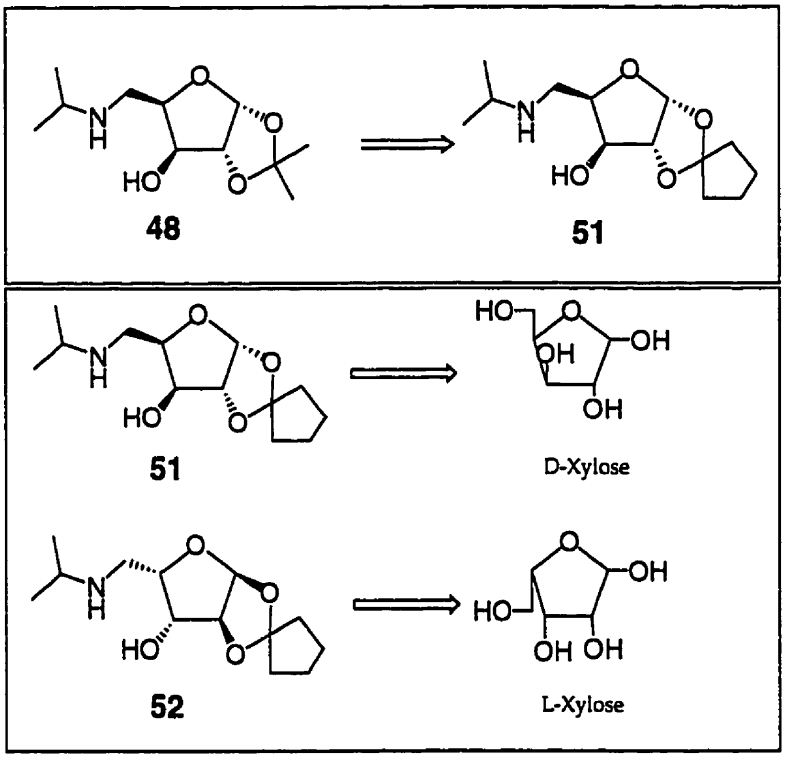
To retain the idea of using a six-membered ring containing the N-P-O part of the phosphoramidite precursor to orient the stereochemistry of the phosphorus center, Biancotto, in our research group, found that using a rigid and bulky chiral γ-aminoalcohol **48** as chiral auxiliary could lead to only one diastereomer of a phosphoramidite **49** without isolation (Scheme 2.3). This preliminary study encouraged us to further investigate the possibility of using the phosphoramidite approach for the stereoselective synthesis of phosphorothioates.



Scheme 2.3: Towards the Stereoselective Synthesis of Phosphorothioates by Using the Phosphoramidite Approach (2)

Considering the removal of the chiral auxiliary at the end of synthesis, we reasoned that the chiral auxiliary **51** with more acid-labile cyclopentylidene group should be more easily removed than the chiral auxiliary **48** with isopropylidene group (Scheme

2.4). The starting material xylose necessary to synthesize this chiral auxiliary **51** is inexpensive, and readily available as both enantiomers. Therefore, with these chiral auxiliaries **51** and **52**, Sp and Rp phosphorothioate dimers have been synthesized as discussed in the following Section 2.3.1 and 2.3.2.

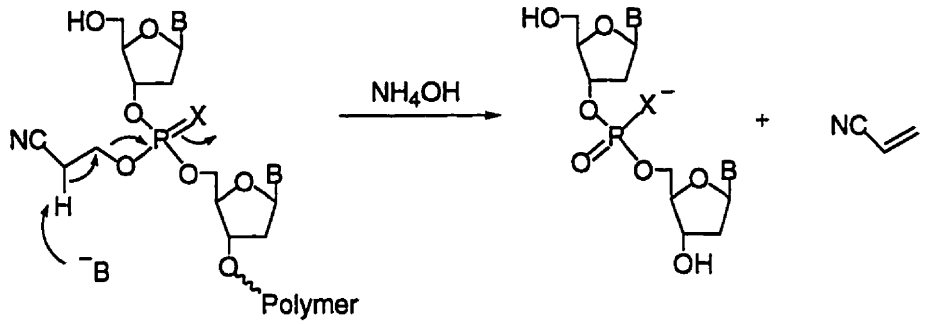


Scheme 2.4: Chiral Auxiliary

In order to improve the reaction conditions in the critical coupling step and then adapt this method to the solid phase synthesis of chiral phosphorothioates, the mechanism of the coupling reaction is discussed as explained in Section 2.3.3. Furthermore, the effect of the acidity of the catalyst on the stereoselectivity and the rate of the coupling reaction will also be discussed in Section 2.3.4.

The commonly used deprotection in normal DNA synthesis is an ammonium hydroxide mediated β-elimination of a cyanoethyl phosphate triester **53** (see Scheme 2.5). The acidity of the proton at the α position to the cyano group and the leaving ability of the

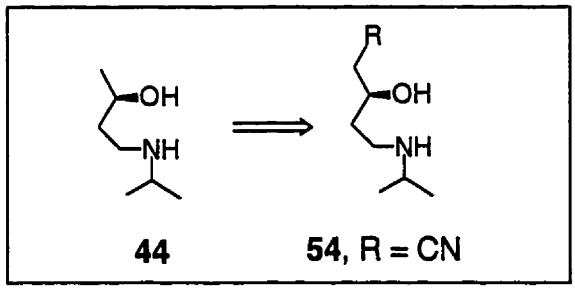
phosphate group make β-elimination possible with a weak base such as ammonium hydroxide in a short time.



53 X = O, S X = O, S

Scheme 2.5: β-Elimination of a Cyanoethylphosphate Triester

Therefore, we tried to synthesize the chiral auxiliary **54** (Scheme 2.6) which could be easily removed at the end of synthesis by base and where the R group could also be modified. A detailed discussion of the studies will be given in Section 2.3.5.



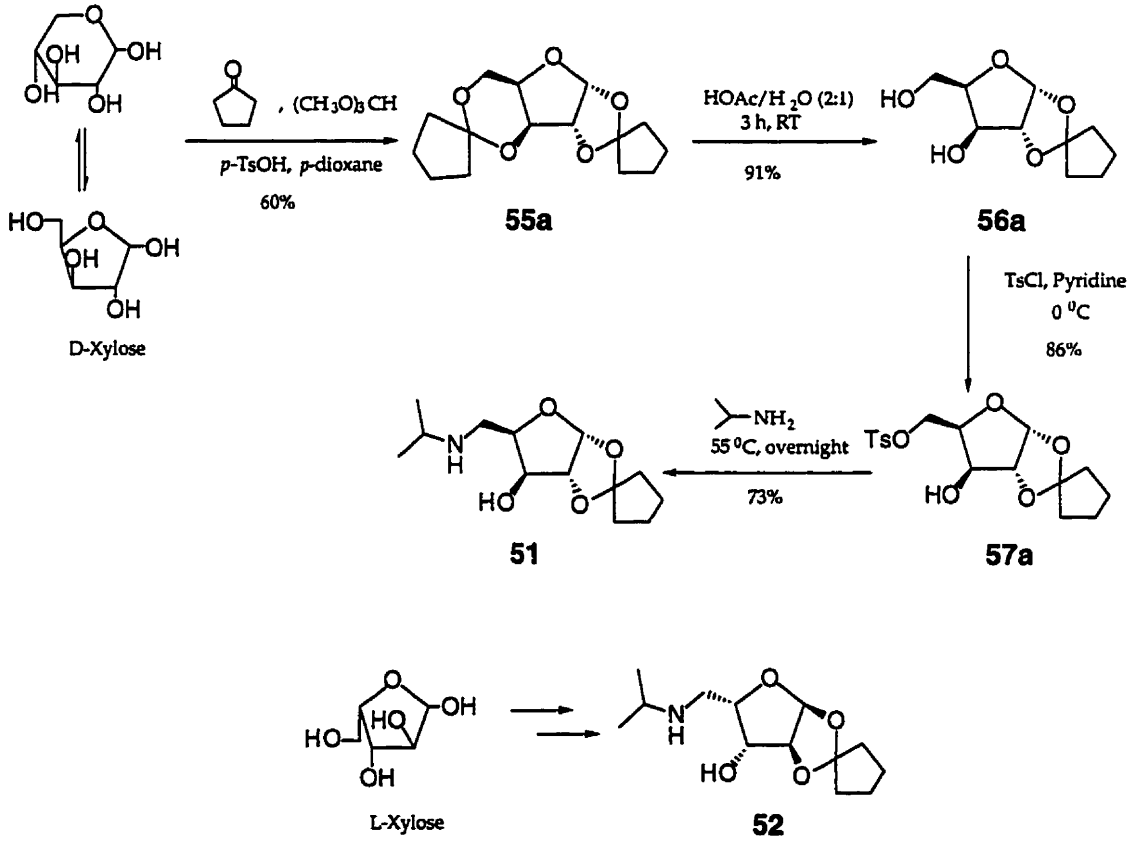
44 **54**, R = CN

Scheme 2.6: Chiral Auxiliary

2.3. Results and Discussion

2.3.1. The Synthesis of Chiral Auxiliaries 51 and 52

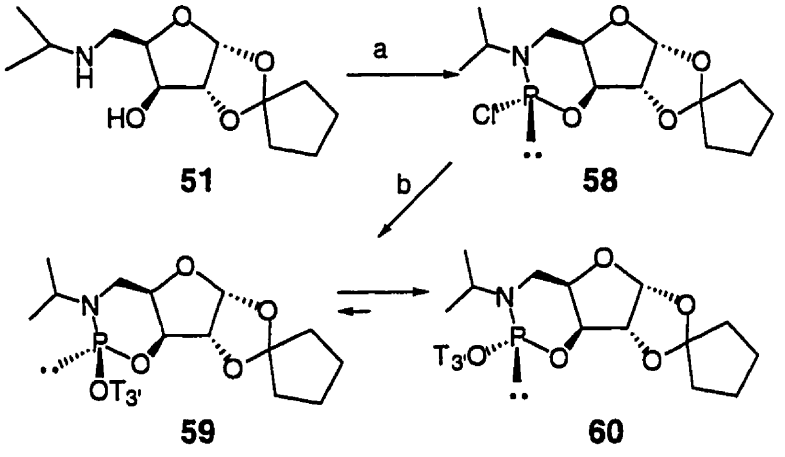
Reaction of D-xylose with a mixture of cyclopentanone and trimethyl orthoformate in the presence of *p*-toluene sulfonic acid in *p*-dioxane[9] gave 1,2-di-O-3,5-di-O-dicyclopentylidene-D-xylofuranose **55a**. Stirring **55a** in acetic acid-water for 3 h at RT gave diol **56a**. Selective tosylation was achieved by treating a 0.1 M pyridine solution of **56a** with a 40% excess of *p*-toluenesulfonyl chloride. Tosylate **57a** was then heated in a ten-fold excess of isopropylamine at 55 °C overnight to provide amine **51**, m.p. 44-45 °C, $[\alpha]^{20}_D = +31.06°$ (c = 2, $CHCl_3$). The overall yield for the transformation D-xylose to **51** was ~60% (Scheme 2.7).



Scheme 2.7: The Synthesis of Chiral Auxiliaries **51** and **52**

In a parallel run, L-xylose was transformed to the enantiomer **52**, m.p. 39-41 °C, $[\alpha]^{20}_D = -31.37^o$ (c = 2, $CHCl_3$) (Scheme 2.7).

2.3.2. The Stereoselective Synthesis of Dithymidine Phosphorothioates



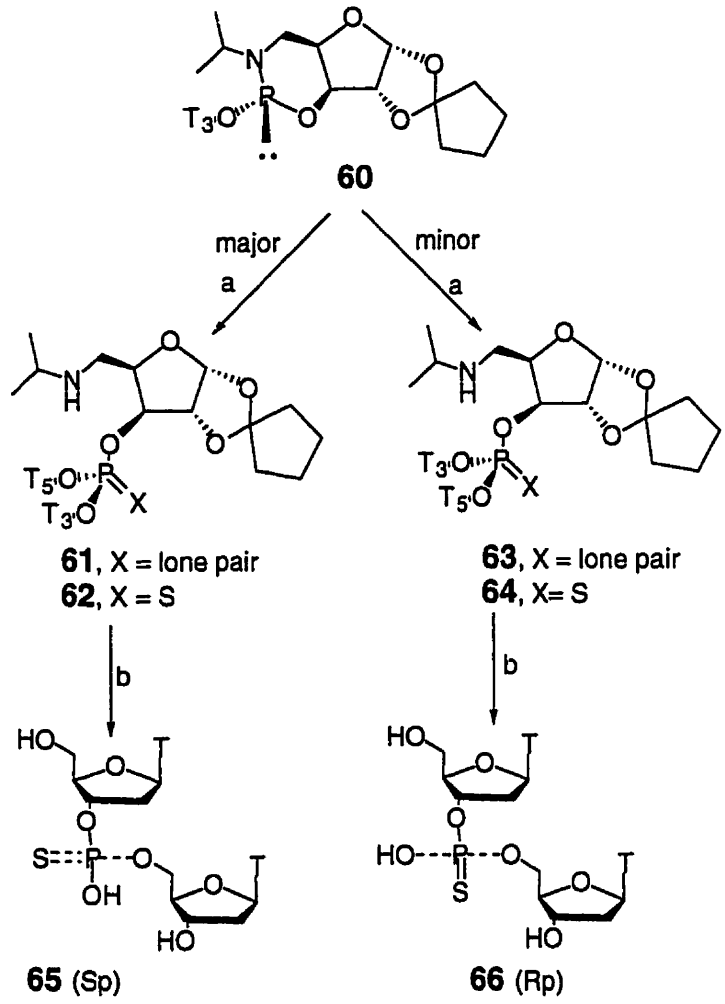
(a) PCl_3, NEt_3, $CHCl_3$, 0 °C - 50 °C.
(b) $T_{3'}OH$, NEt_3, $CHCl_3$, 0 °C - 50 °C.

Scheme 2.8: The Synthesis of Diastereomerically Pure Phosphoramidite **60**

Reaction of γ-aminoalcohol **51** with PCl_3 and triethylamine in dry chloroform provided phosphorochloridite **58**. Its ^{31}P NMR showed a single peak at 148.42 ppm. By analogy with phosphoramidite **60**, it seemed that it had most likely the Rp stereochemistry as depicted in Scheme 2.8. Reaction of the phosphorochloridite **58** with 5'-O-*tert*-butyldimethylsilylthymidine (**$T_{3'}OH$**) in dry chloroform gave a mixture of phosphoramidites **60** and **59** (^{31}P NMR 129.80 ppm and 138.70 ppm respectively) in a ratio which was time and temperature dependent. At -78 °C, the two isomers were formed in a 3:2 ratio, whereas at RT, the ratio was > 10 : 1. After overnight heating at 50 °C, the

minor isomer **59** corresponding to the peak at 138.70 ppm was quantitatively isomerized to the thermodynamically more stable isomer **60** (129.80 ppm). Both isomers were configurationally stable after extraction and removal of triethylammonium chloride and not separable by flash chromatography.

The coupling reaction between phosphoramidite **60** and 3'-O-*tert*-butyldimethylsilyl thymidine ($\mathbf{T_{5'}OH}$) was first carried out at room temperature in acetonitrile, using 2-bromo-4,5-dicyanoimidazole **46** as catalyst. It provided phosphite triesters **61** and **63** in a ratio of 6:1, as established by ^{31}P NMR (143.76 and 142.55 ppm for the major and minor isomer respectively). Without purification, the mixture of triesters was sulfurized with Beaucage's reagent[10] to give a 6:1 mixture of phosphorothioates **62** and **64** (^{31}P NMR 68.23 and 68.43 ppm respectively). When a similar coupling reaction was carried out in $CHCl_3$ at 0 ^{0}C for 4 h and at -15 ^{0}C for 6 h, the ratio of **62** to **64** increased to 40:1 and 68:1, respectively. Chromatography of the reaction mixture obtained at -15 ^{0}C provided one isomer of **62** only. After hydrolysis of **62/64** with 70% trifluoroacetic acid at RT, the phosphorothioate dinucleosides **65** (^{31}P NMR 55.70 ppm) and **66** (^{31}P NMR 56.10 ppm) were obtained in 85% yield with an Sp : Rp diastereomer ratio the same as the ratio of **62** to **64** (Scheme 2.9). The configurations of the major diastereomer **65** and minor diastereomer **66** were established as Sp and Rp, respectively, by snake venom phosphodiesterase, P1 nuclease digestion and also by HPLC analysis.[11] It was opposite to the one we had tentatively and erroneously assigned[12] because the chemical shifts of ^{31}P NMR for the phosphorothioate dinucleosides **65** and **66** were opposite in CD_3OD and D_2O. In D_2O, the signal of ^{31}P NMR corresponding to the Sp diastereomer occurred at higher field (^{31}P NMR 55.70 ppm) than that of the Rp diastereomer (^{31}P NMR 56.10 ppm)[13], while in CD_3OD the signal of ^{31}P NMR corresponding to the Sp diastereomer occurred at lower field (^{31}P NMR 58.64 ppm) than that of the Rp diastereomer (^{31}P NMR 58.57 ppm).

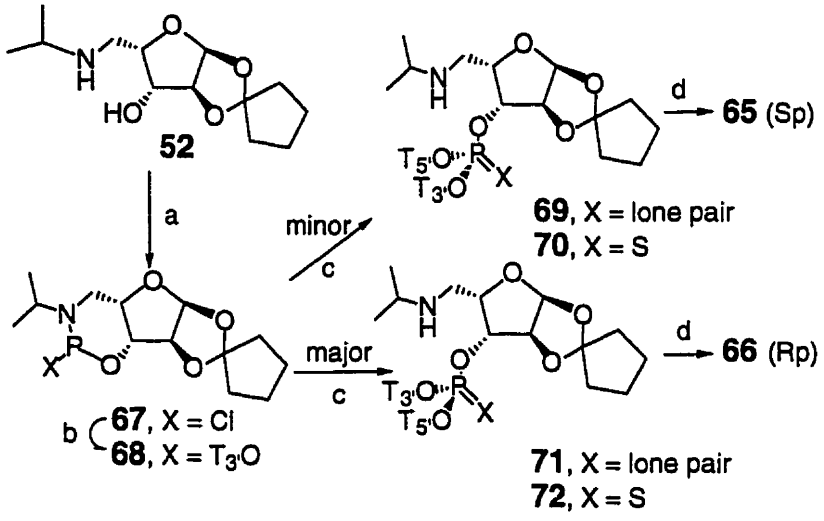


(a) $T_{5'}OH$, 2-bromo-4,5-dicyanoimidazole, CH_3CN or $CHCl_3$; then Beaucage's reagent. (b) 70% TFA, RT

Scheme 2.9: The Synthesis of Sp Dithymidine Phosphorothioate

In a series of reactions identical to those described (Scheme 2.8 and Scheme 2.9), **52** was converted via phosphorochloridite **67** (^{31}P NMR 148.75 ppm) to phosphoramidite **68** (^{31}P NMR 129.34 ppm). Phosphoramidite **68** then underwent the coupling reaction with $T_{5'}OH$ to give **69** and **71** which were then sulfurized with Beaucage's reagent to give **70** and **72** (^{31}P NMR 68.91 and 69.12 ppm respectively). The diastereomeric ratio of **70** and **72** was 1:7 when the coupling reaction was performed

at RT in acetonitrile. When the coupling reaction was performed at -15 ^{0}C in chloroform, the diastereomeric ratio of **70** and **72** was 1:70 as determined by ^{31}P NMR. Only isomer **72** could be isolated by chromatography. After hydrolysis of **70/72** with 70% TFA at RT, the phosphorothioate dinucleosides **65** and **66** were obtained with an Sp : Rp diastereomeric ratio identical to that of **70** to **72** (Scheme 2.10).



(a) PCl_3, NEt_3, $CHCl_3$, 0 ^{0}C - 50 ^{0}C. (b) **$T_{3'}$OH**, NEt_3, $CHCl_3$, 0 ^{0}C - 50 ^{0}C. (c) **$T_{5'}$OH**, 2-bromo-4,5-dicyanoimidazole, CH_3CN or $CHCl_3$; then Beaucage's reagent. (d) 70% TFA, RT

Scheme 2.10: The Synthesis of Rp Dithymidine Phosphorothioate

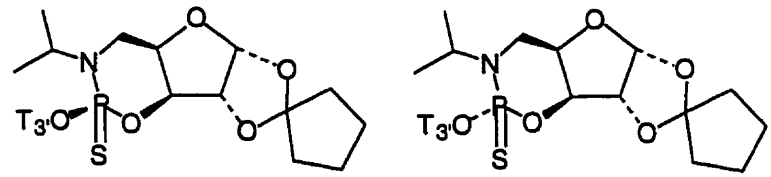
In summary, the use of chiral auxiliary **51** derived from D-xylose led to phosphorothioate dinucleoside **65** (Sp), while use of the chiral auxiliary **52** derived from L-xylose led to phosphorothioate dinucleoside **66** (Rp).

2.3.3. The Mechanism of the Coupling Reaction

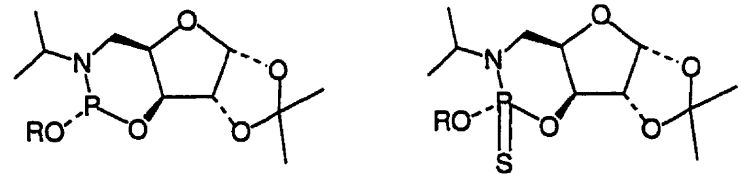
The essential step in the synthesis of the phosphite triester and therefore of phosphorothioate is the azole-activated (tetrazole or, in our case, 2-bromo-4,5-

dicyanoimidazole) coupling reaction of the phosphoramidite with the free 5'-hydroxyl function of thymidine. The mechanism of this activation process still remains to be clarified. The main question is whether azole serves only as a proton donor yielding as primary product a protonated nucleoside phosphoramidite or whether it also attacks as a nucleophile giving rise to the formation of an azolide intermediate.[6] In the first case, after protonation of phosphoramidite, the free 5'-hydroxyl group of thymidine ($\mathbf{T_5'OH}$) reacts with inversion of configuration at the phosphorus center to give the desired phosphite triester. The second assumption involves the displacement of the amine function of the amidite by the azole after protonation, followed by either displacement of the azole by another azole leading to a diastereomeric mixture of phosphorazolides or by displacement of the azole by an alcohol which gives the desired phosphite triester. The phosphite triesters are then sulfurized. The latter reaction is known to proceed with retention of configuration.[14]

The stereochemistry of a reaction is one important clue to the reaction mechanism. With structure assignments for the intermediate phosphoramidites **59** and **60** being somewhat ambiguous, we first tried to obtain crystals of phosphoramidite **60** or of the corresponding thioate **60S** (^{31}P NMR 67.5 ppm) or **59S** (^{31}P NMR 72.4 ppm)[15] for X-ray crystallographic structure determination. None of them gave suitable crystals. The same was also true for phosphoramidites **73a-d** (^{31}P NMR 129.0, 128.2, 125.3 and 127.5 ppm, respectively), in which the exocyclic thymidine was replaced by isopropanol, cholesterol, and *p*-nitrophenol, and for their sulfur derivatives **74a-d** (^{31}P NMR 67.5, 66.6, 62.0, and 66.8 ppm respectively) (Figure 2.1). The synthesis of phosphoramidites **73a-d** and the corresponding thioates **74a-d** is shown in Scheme 2.11.



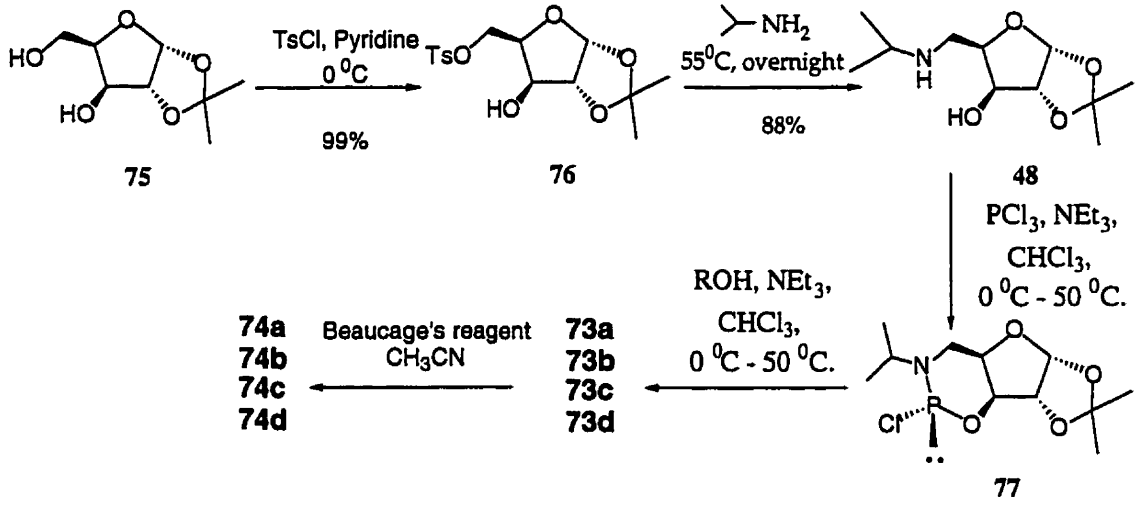
59S **60S**



73a: R = $OT_{3'}$
73b: R = isopropyl
73c: R = *p*-nitrophenyl
73d: R = cholesteryl

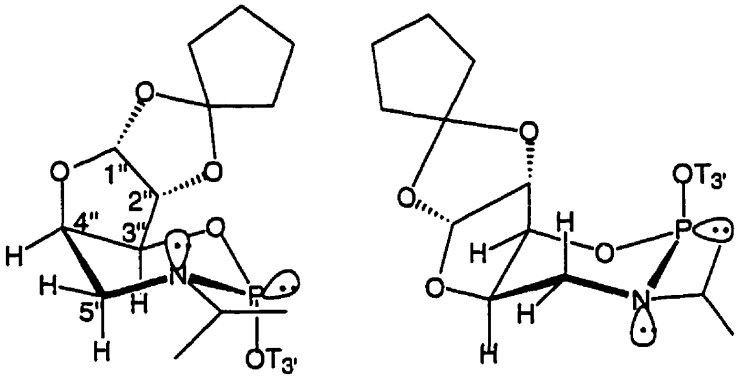
74a: R = $OT_{3'}$
74b: R = isopropyl
74c: R = *p*-nitrophenyl
74d: R = cholesteryl

Figure 2.1: The Structures of Phosphoramidites and the Corresponding Thioates



Scheme 2.11: The Synthesis of Phosphoramidites **73a-d** and the Corresponding Thioates **74a-d**

The structural assignment for the phosphoramidites **59** and **60** was therefore made as described by Huang *et al.* and others.[16,17,18,19] They showed that in bicyclic six-membered ring phosphites, the 1,3,2-dioxaphosphorinane ring preferentially adopted a chair conformation with the exocyclic OR attached to the phosphorus group in an axial position. The thermodynamically less stable isomers, however, showed a preference for a twist conformation of the dioxaphosphorinane ring in which the OR group occupied a pseudoaxial position[16] instead of a chair conformation in which the OR group would occupy an equatorial position. By analogy, we suggested that the thermodynamically more stable phosphoramidite **60** had the thymidine residue at the axial position of the six-membered ring, which had a chair conformation (Figure 2.2).



60a **60b**

Figure 2.2: The Conformation of Phosphoramidite **60**

The axial position of $OT_{3'}$ was based on the ^{13}C NMR data: the large ^{2}J coupling constant between the $C_{3'}$ of thymidine and phosphorus of 19.2 Hz, the analogous coupling of 3.7 Hz for C-O-P of the oxazaphosphorinane carbon, and the small coupling constant of 1.8 Hz for $J_{C4''-P}$ in isomer **60** (Table 2.1).

Table 2.1: Selected ^{13}C NMR Coupling Constants *

comp.	$J_{C3'-P}$	$J_{C2''-P}$	$J_{C3''-P}$	$J_{C4''-P}$	$J_{C5''-P}$	J_{CHN-P}
59S	4.6	12	6.3	1.8	5.5	6.4
60S	5.5	11	9.2	4.6	7.3	6.4
59	10	4.6	2	2.7	3.7	37.5
60	19.2	4.6	3.7	1.8	3.2	36.6

* Solvent: $CDCl_3$. The carbons of the branched-chain sugar units at the 3'-end of the molecule are denoted by C' and the carbons of the sugar at the chiral auxiliary are denoted by C''.

Therefore, there are only two possible conformers **60a** (Sp) and **60b** (Rp) for the isomer **60** (Figure 2.2), of which **60a** should be strongly favored because of the far smaller 1,3-diaxial interactions of the P-OT_3. bond.

The conformational analyses of the six-membered rings were also based on the values of $^3J_{POCH}$ and $^3J_{HCCH}$. It should be noticed that coupling constants $^3J_{PNCH}$ have not been found to be useful in conformational analyses of three-coordinate phosphorus-containing heterocyclic six-membered rings.[16,19]

The data observed for conformer **60a** are similar to those found in the previously studied three-coordinate 1,3,2-dioxa- and oxazaphosphorinanes that populate analogous chair conformations with substituents attached axially to phosphorus. Thus the small values of $^3J_{H3''-P}$ (1.0 Hz), $^3J_{H3''-H4''}$ (2.4 Hz), $^3J_{H4''-H5a''}$ (2.4 Hz), $^3J_{H4''-H5e''}$ (2.4 Hz), and $^3J_{H5a''-P}$ (2.4 Hz) recorded are characteristic of gauche HCCH and HCOP arrangements, while the large value of $^3J_{H5e''-P}$ (7.1 Hz) observed is typical of the antiperiplanar HCOP geometry (Table 2.2).

Table 2.2: Selected ^{1}H NMR Coupling Constants *

comp.	$J_{H3''-H4''}$	$J_{H3''-P}$	$J_{H4''-H5''a}$	$J_{H4''-H5''e}$	$J_{H5''a-P}$	$J_{H5''e-P}$	$J_{H3'-P}$
59S	4.1	5.3	6.6	6.4	12	19	10
60S	2.4	2.4	2.4	2.4	3.2	20	10
59	3.9	3.2	7.3	7.3	4.7	6.1	9.0
60	2.4	1.0	2.4	2.4	2.4	7.1	10.5

* Solvent: $CDCl_3$. The protons of the branched-chain sugar units at the 3'-end of the molecule are denoted by H' and the protons of the sugar at the chiral auxiliary are denoted by H''.

The conformation of **60** was also confirmed by a 2D NOE experiment in which $H_{5''a}$ showed NOE with the $H_{3''}$. The 2D NOE experiment also showed a cross peak between $H_{3''}$ and $H_{4'}$ which tells us that $H_{3''}$ and $OT_{3'}$ should be on the same side of the six-membered ring. No detectable NOE with $H_{2''}$ / $H_{5''a}$ or $H_{1''}$ / $H_{5''a}$ was observed.

Using a similar line of reasoning, twist conformation **59c** is the most likely conformation for **59** (Figure 2.3).

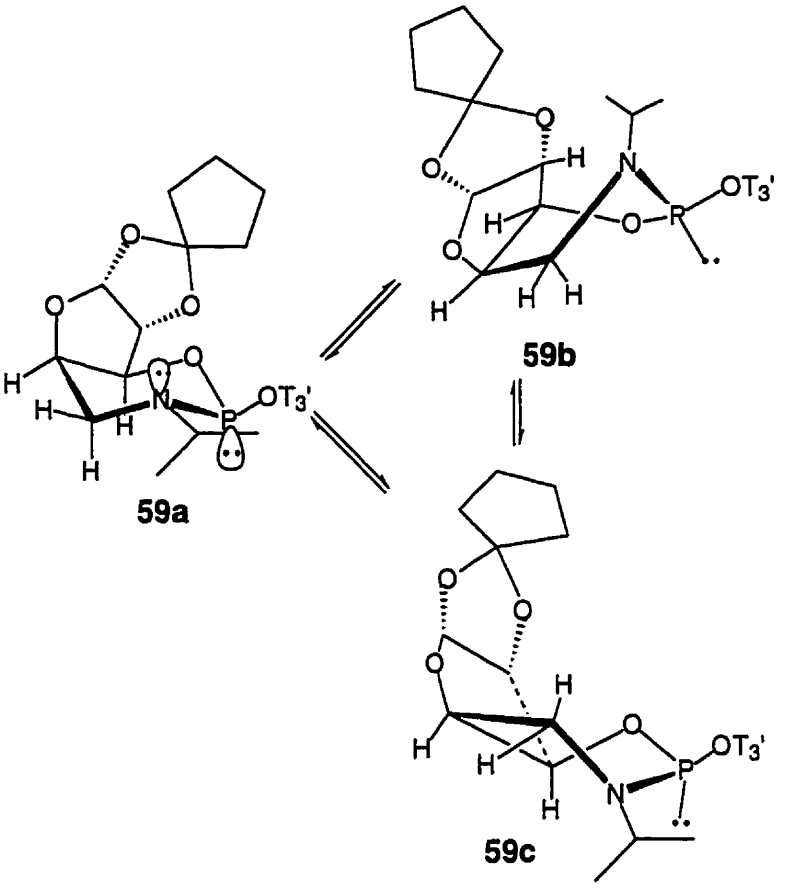
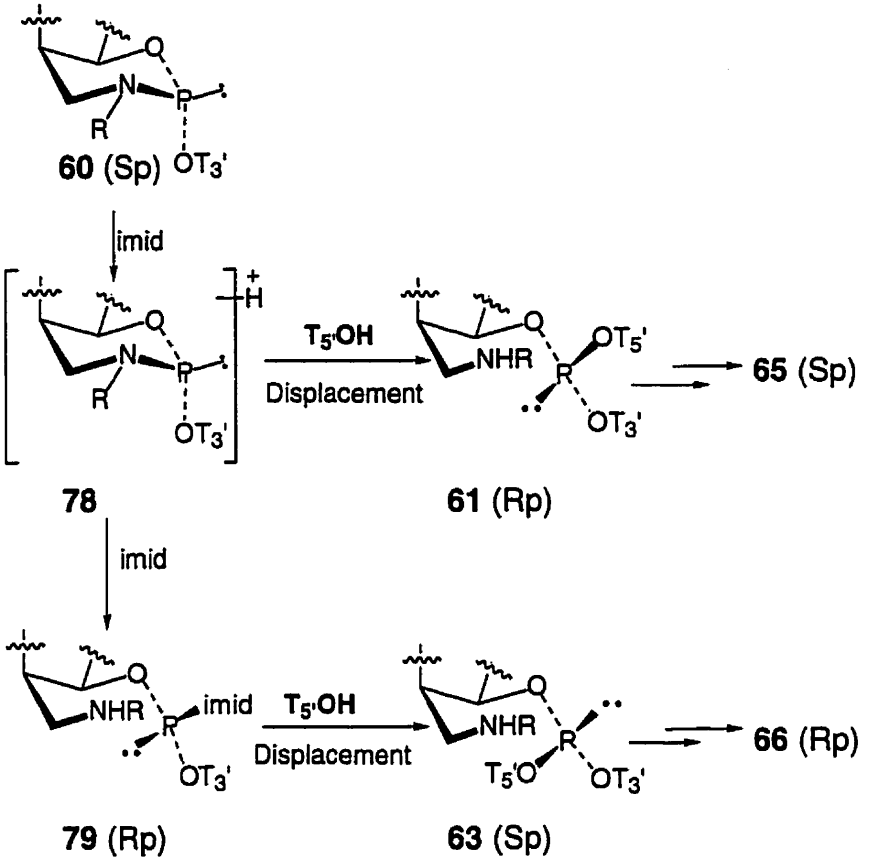


Figure 2.3: The Conformation of Phosphoramidite **59**

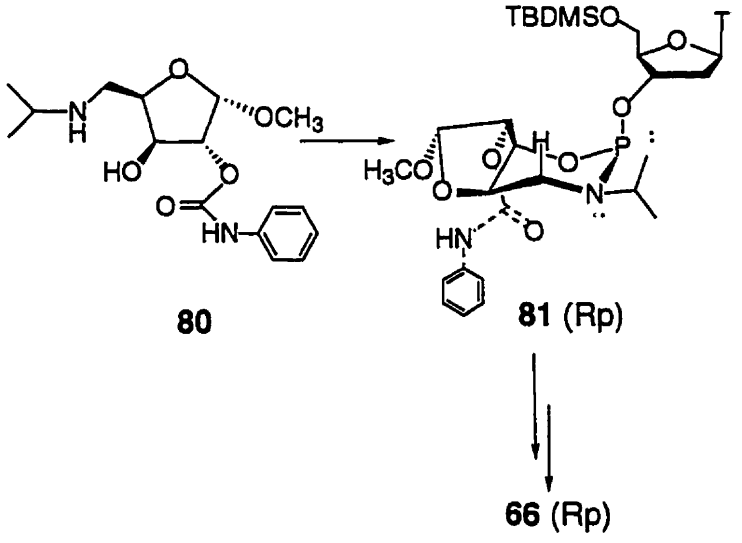
When we used a 3:2 mixture of **60** and **59** to carry out the coupling reaction with thymidine derivative $\mathbf{T_5OH}$ under the same coupling reaction conditions as for diastereomer **60**, we obtained the phosphite triesters **61** and **63** in a ratio of 3:2 (see Scheme 2.9). Treatment with Beaucage's reagent gave a 3:2 mixture of phosphorothioates **62** and **64** (^{31}P NMR 68.23 and 68.43 ppm respectively). We could therefore conclude that the two isomers gave reversed diastereoselectivity in the coupling reaction, with **59** giving a phosphorothioate with the Rp configuration. We also observed that the thermodynamically less favored diastereomer **59** was considerably more reactive than diastereomer **60**.



Scheme 2.12: The mechanism of the coupling reaction. (imid = 2-bromo-4,5-dicyanoimidazole or its anion, R = isopropyl)

Analysis of the stereochemistry of phosphoramidite **60** (Sp) and phosphorothioate **65** (Sp) indicated that the transformation involved one inversion only for this particular system (Scheme 2.12). We therefore thought the possible mechanism for the coupling reaction was: after protonation of phosphoramidite, the nucleoside (**T_5'OH**) directly attacked the phosphorus center of **78** and gave Rp phosphite triester **61**. This excluded the double inversion postulated by Stec and Zon[4] and by Seliger *et al.*[6] Indeed, when bromoacetic acid (pKa = 2.86) was used as activator in the coupling reaction, the same phosphite triesters **61** and **63** were obtained in a ratio of 4:1. This was to be compared to a 6:1 ratio when 2-bromo-4,5-dicyanoimidazole was used as catalyst, using identical reaction conditions. Furthermore, when the proton of the catalyst 2-bromo-4,5-

dicyanoimidazole **46** was blocked by reacting with diisopropylamine, there was no coupling reaction between phosphoramidite **60** and nucleoside (T_5,**OH**). This result further confirmed the mechanism involving a protonation process. The use of a closely related chiral auxiliary **80** gave the opposite Rp stereochemistry (Scheme 2.13).[20] If the mechanism we suggested here was general, the phosphoramidite **81** formed from chiral auxiliary **80** should have opposite configuration (Rp).



Scheme 2.13: The Synthesis of the Rp Dithymidine Phosphorothioate

2.3.4. The Effect of the Acidity of the Catalyst on the Stereoselectivity and the Rate of the Coupling Reaction

When trying to apply the 2-bromo-4,5-dicyanoimidazole-catalyzed coupling reaction of the phosphoramidite to 5'-dimethoxytritylthymidine, we realized that the reaction conditions were too acidic, resulting in hydrolysis of the dimethoxytrityl group. The same was true when the thymidine was substituted at 5' with a monomethoxytrityl function. A trityl group appeared to be stable but is not suitable for automated DNA

synthesis. We therefore investigated other less acidic catalysts to see if 2-bromo-4,5-dicyanoimidazole could be replaced without loss of stereoselectivity of the reaction.

First, we tried to examine the pKa effect of catalyst. Since 1H-tetrazole (pKa = 4.8) is a commonly used efficient activator for the coupling reactions,[6] we tried using it in our coupling reaction. ^{31}P NMR showed the coupling reaction to be very slow and without good stereoselectivity. We also tried other catalysts with pKa > 4.8, such as 4,5-dicyanoimidazole **82** (pKa = 5.2), 2-benzyl-4,5-dicyanoimidazole **83** (pKa = 5.3), 2-bromo-4,5-diethylcarboxylimidazole **84** (pKa = 6.4) and 2-nitroimidazole **85** (pKa = 6.4). The ^{31}P NMR showed that the coupling reactions were very slow and without stereoselectivity. Then we tried using a catalyst with pKa < 4.8. Benzimidazolium triflate **86** (pKa = 4.5) and *p*-nitrophenyltetrazole **87** (pKa = 3.7) have been reported to be better activators than 1H-tetrazole,[21] but the coupling reaction was slow and without stereoselectivity in our system.

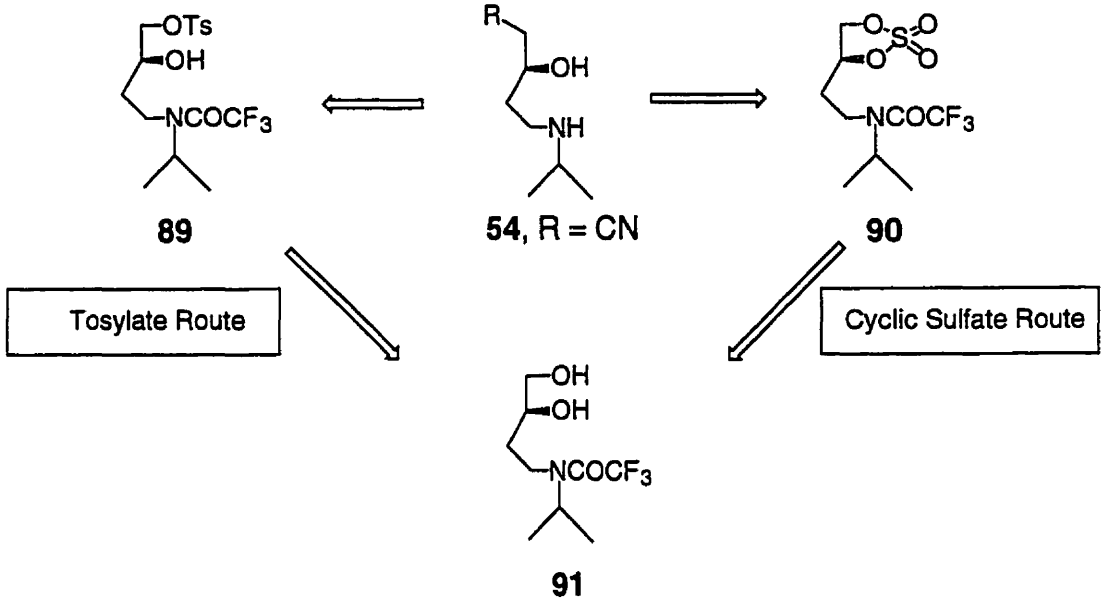
Since increasing the size of the substituents of imidazole seemed to give higher stereoselectivity,[8a] we synthesized 2-iodo-4,5-dicyanoimidazole **88** and found that it did not give better stereoselectivity than 2-bromo-4,5-dicyanoimidazole **46**. Also it is not a good catalyst choice because of its high acidity.

2.3.5. Work Towards the Synthesis of Chiral Auxiliary for Chiral Phosphorothioates

As discussed in Section 2.2.3, a phosphoramidite based method which can be adapted to the stereoselective synthesis of phosphorothioates requires the design of a new chiral auxiliary. Since the xylose approach required strong acid to remove chiral auxiliary, a synthesis of a chiral auxiliary which could be removed by base was also undertaken. The chiral auxiliary **54** was chosen as a candidate, since it can also retain the idea of using a

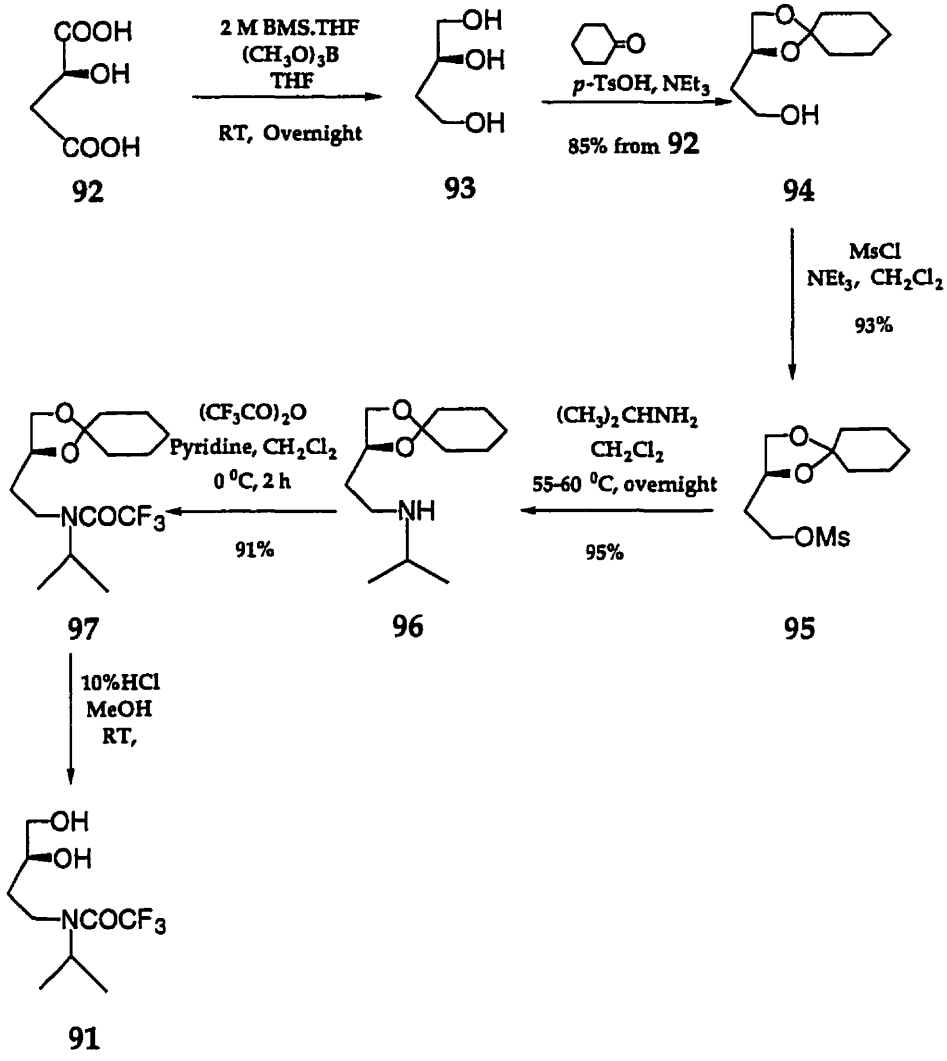
six-membered ring containing the N-P-O part of the phosphoramidite precursor to orient the stereochemistry of the phosphorus center.

Scheme 2.14 shows two routes which we have tried.



Scheme 2.14: The Retrosynthesis of Chiral Auxiliary **54**

The synthesis of precursor **91** started from cheap, commercially available L-malic acid **92** which has already possessed a chiral center. Quantitative reduction of L-malic acid **92** with borane-dimethylsulfide and trimethylborate afforded 1,2,4-butanetriol **93**.[22] The protection at the 1,2 positions of this triol was easily performed by treating it with cyclohexanone in presence of *p*-toluenesulfonic acid. Flash chromatography then afforded the desired product **94**. The remaining free hydroxyl group of **94** was mesylated with mesyl chloride in dichloromethane and triethylamine to give mesylated compound **95**. The mesylate group of **95** was then displaced by isopropylamine to form **96**, followed by protection of this amine group with trifluoroacetic anhydride to give **97**. Finally, the acetal protecting group of **97** was removed by acidic treatment to furnish the diol **91** (Scheme 2.15).

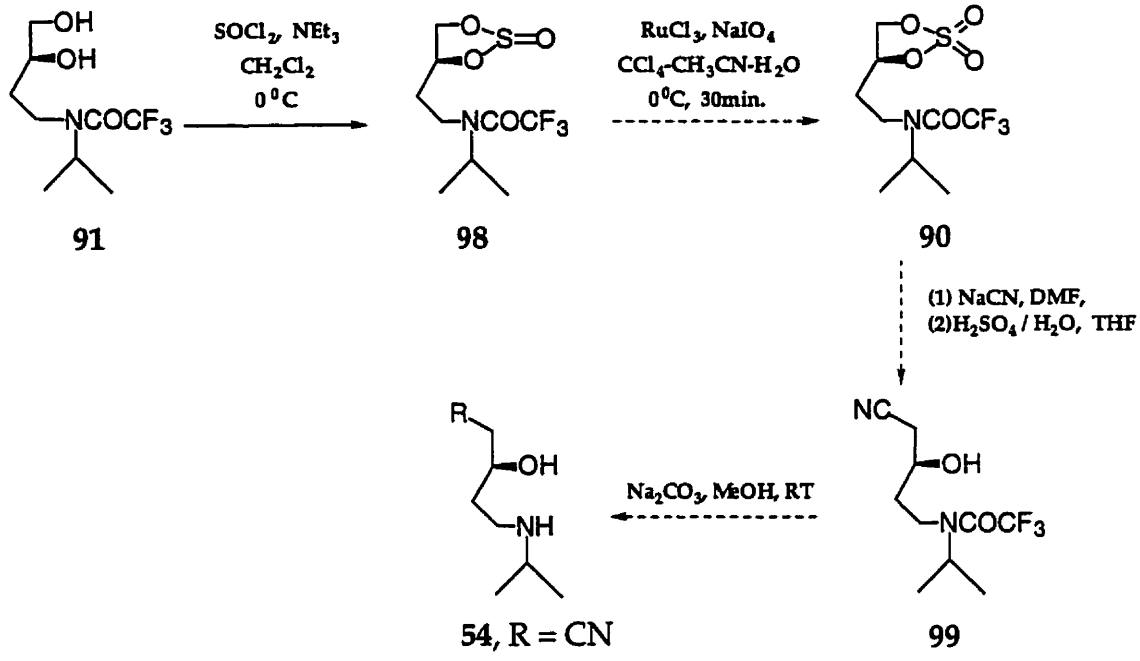


Scheme 2.15: The Synthesis of Precursor **91**

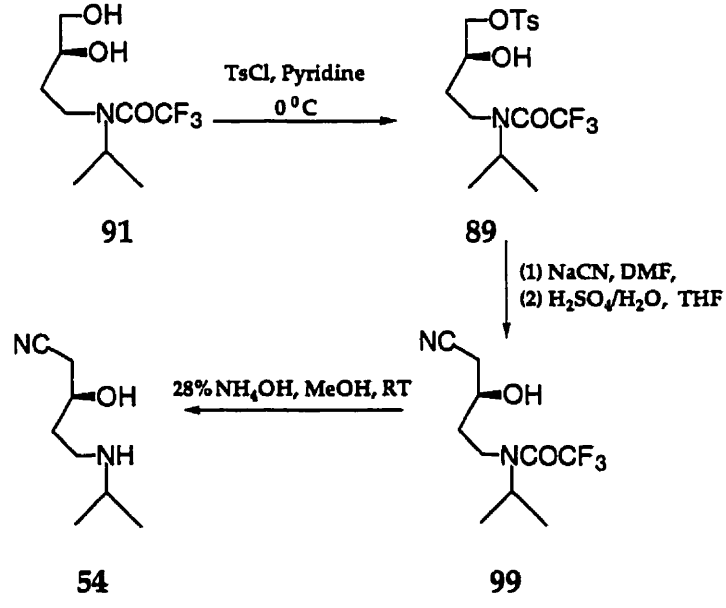
We next attempted to form a cyclic sulfate **90**, which is a highly reactive epoxide equivalent. Diol **91** reacted with thionyl chloride to give cyclic sulfite **98**, but the oxidation of the sulfide **98** did not form cyclic sulfate **90** (Scheme 2.16). A literature survey showed that acyclic 1,2-diols did not yield cyclic sulfates.[23]

Therefore, the diol **91** was tosylated to give tosylate **89**. Reaction with sodium cyanide gave the cyano derivative **99** which was hydrolyzed to furnish the desired chiral auxiliary **54** (Scheme 2.17).

This route was not further investigated due to time constraints.



Scheme 2.16: The Cyclic Sulfate Route to the Chiral Auxiliary **54**



Scheme 2.17: The Tosylate Route to the Chiral Auxiliary **54**

2.4. Conclusion

We have shown that diastereomerically pure cyclic phosphoramidites **60** and **68** obtained without chromatographic purification can lead diastereoselectively to Sp and Rp dithymidine phosphorothioates **65** and **66** respectively. The conditions employed here were suitable for solution synthesis but are for the time being not applicable to solid phase synthesis. The stereochemistry of the coupling reaction suggested only one inversion at phosphorus in the case of 1,2-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **51** or 1,2-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-L-xylofuranose **52** derived phosphoramidites, if our analysis of the conformers of the intermediate phosphoramidites is correct. It should however be noticed that a closely related system described in Scheme 2.13 gives the opposite stereochemistry.

2.5. Experimental

2.5.1. General Materials and Methods

^{1}H NMR spectra were recorded on Varian XL200 or Unity 500 Spectrometer. ^{13}C NMR spectra were determined at 50 MHz and 125.7 MHz on Varian XL200, or Unity 500 spectrometer. ^{1}H and ^{13}C NMR chemical shifts are expressed in ppm relative to the internal tetramethylsilane(TMS). ^{1}H NMR peak assignments for all the compounds were derived from 2D-COSY and in special cases NOE (compounds **59**, **60**, **59S**, **60S**, **68**) experiments. ^{13}C NMR peak assignments for all compounds are derived from 2D-HMQC experiments. ^{31}P NMR spectra were taken on a Varian XL 300, UNITY 500 spectrometers at 121 and 202 MHz with proton-decoupling ($\{^{1}H\}$). Positive ^{31}P NMR chemical shifts are expressed in ppm downfield from external 85% H_3PO_4. Spin multiplicites are given with the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad peak.

Low resolution mass spectra were recorded on a KRATOS MS 25RFA mass spectrometer in the direct-inlet mode. High resolution mass spectra were recorded on a ZAB 2F HS mass spectrometer.

Optical rotation measurements were measured on a JASCO DIP-140 digital polarimeter using a 10 mm length cell at the indicated wavelength, temperature and concentrations (calculated in g/100 ml solvent).

Melting points (m.p.) were determined on a Gallenkamp block and are uncorrected.

Dichloromethane and chloroform were distilled from P_2O_5, methanol from magnesium, triethylamine and acetonitrile from CaH_2 and THF from sodium benzophenone ketyl. Pyridine was refluxed for 4 hours with fine BaO and then distilled over granular BaO under N_2. Anhydrous DMF was purchased from Aldrich in sure-seal bottles and used with no further drying. Tetrazole and Beaucage's reagent were gifts from Isis Pharmaceuticals. All other chemicals were purchased from Aldrich Chemical Company Inc. and were used without further purification.

Thin-layer chromatography (TLC) was performed using Kieselgel 60 F_{254} aluminum backed plates (0.2 mm thickness). Column chromatography was performed on 230-400 mesh silica gel (Merck).

All air sensitive experiments were performed under dry argon with freshly distilled anhydrous solvents and glassware previously dried overnight in an oven.

2.5.2. Experimental for Section 2.3.1.

1,2-O-3,5-O-dicyclopentylidene-α-D-xylofuranose (55a).

To a solution of trimethylorthoformate (2.74 ml, 25 mmol) and *p*-toluene sulfonic acid (190 mg, 1.0 mmol) in dioxane (50 ml) under a nitrogen atmosphere at 0 ^{o}C, was added dropwise cyclopentanone (17.5 ml, 0.2 mol). This solution was stirred at RT for 2 h and D-xylose (1.5 g, 10 mmol) was added with stirring continued overnight. Then the reaction mixture was neutralized by triethylamine. The solvent was then evaporated to furnish a

yellow syrup. A solution of the syrup residue in chloroform was washed with water and the aqueous layer was then extracted with chloroform three times. The combined chloroform layers were dried over $MgSO_4$. After removing the solvent, the mixture was chromatographed on a silica gel column (Hexane:Ethyl acetate = 5:1) to give a white solid **55a** (1.7 g, 60%): m.p. 85 - 87 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.99 (d, J = 3.8 Hz, 1H, H-1), 4.45 (d, J = 3.8 Hz, 1H, H-2), 4.24 (d, J = 2.2 Hz, 1H, H-3), 4.14 - 3.46 (m, 3H, H-4, 2 x H-5), 1.98 - 1.55 (m, 16H, cyclopentylidene protons); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.23 (C-1O<u>C</u>OC-2), 109.24 (C-3O<u>C</u>OC-5), 105.08 (C-1), 84.49 (C-2), 74.25 (C-4), 71.83 (C-3), 61.50 (C-5), 39.52, 36.82, 36.19, 29.74, 24.09, 23.49, 22.80, 22.37 (cyclopentylidene carbons); MS (CI) m/e 283 ($M+H^+$).

1,2-O-cyclopentylidene-α-D-xylofuranose (56a).

The 1,2-O-3,5-O-dicyclopentylidene-α-D-xylofuranose **55a** (2.82 g, 10 mmol) was dissolved in a mixture of acetic acid : water (2:1) (140 ml) at RT. The reaction mixture was stirred for 3 h. The reaction was followed by TLC. The solvent was then evaporated under high vacuum, coevaporate with methanol three times and dried under vacuum overnight to give a white solid **56a** (2.16 g, 100%): m.p. 58 - 60 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 (d, J = 3.9 Hz, 1H, H-1), 4.44 (d, J = 3.9 Hz, 1H, H-2), 4.32 (m, 1H, H-3), 4.18 (m, 1H, H-4), 4.10 - 4.02 (m, 2H, 2 x H-5), 1.96 - 1.61 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.41 (OCO), 104.53 (C-1), 85.59 (C-2), 78.76 (C-3), 76.83 (C-4), 61.07 (C-5), 36.84, 36.19 ($\underline{C}H_2C\underline{C}H_2$), 23.47, 22.80 ($\underline{C}H_2CH_2CCH_2\underline{C}H_2$); MS (CI) m/e 217 ($M+H^+$).

1,2-O-cyclopentylidene-5-O-tosyl-α-D-xylofuranose (57a).

To a solution of 1,2-O-cyclopentylidene-α-D-xylofuranose **56a** (1.76 g, 8.1 mmol) in dry pyridine (50 ml) under nitrogen atmosphere at 0 ^{0}C, *p*-toluenesulfonyl chloride (1.73 g,

1.12 eq.) was added. The reaction mixture was stirred at 0 °C for 3 h. Then 50ml of water was added and the solution was evaporated and coevaporated with toluene under vacuum twice. The mixture was dissolved in chloroform, washed with water three times and dried over $MgSO_4$. Removal of the solvent furnished a white solid **57a** (2.99 g, 100%): m.p. 98 - 100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 - 7.32 (AA'BB', 4H, Ph), 5.84 (d, J = 3.9 Hz, 1H, H-1), 4.44 (d, J = 3.9 Hz, 1H, H-2), 4.42 - 4.29 (m, 3H, H-3, 2 x H-5), 4.27 - 4.09 (m, 1H, H-4), 2.44 (s, 3H, CH_3), 1.89 - 1.61 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 145.34, 132.30, 130.06, 128.08 (aromatic carbons), 121.86 (OCO), 104.80 (C-1), 85.15 (C-2), 77.73 (C-3), 74.41 (C-4), 66.21 (C-5), 37.02, 36.44 ($\mathbf{C}H_2\mathbf{C}CH_2$), 23.54, 22.98 ($\mathbf{C}H_2CH_2\mathbf{C}CH_2\mathbf{C}H_2$), 21.73 ($CH_3$); MS (CI) m/e 371 ($M+H^+$).

1,2-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose (51). A solution of 1,2-O-cyclopentylidene-5-O-tosyl-α-D-xylofuranose **57a** (2.64 g, 7.1 mmol) in isopropylamine (15 ml) was stirred at 55 °C overnight in a pressure bottle. The solvent was removed in vacuo and the remaining yellow syrup obtained was taken up with chloroform, washed with saturated $NaHCO_3$, brine, and dried over $MgSO_4$. After removal of the solvent, the mixture was chromatographed on silica gel (ethyl acetate/3% triethylamine) to furnish a white solid **51** (1.33 g, 73%): m.p. 44 - 45 °C; $[\alpha]^{20}_D$ = 31.06 (C = 2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.0 (b, NH), 5.90 (1H, d, J = 3.9 Hz, H-1), 4.38 (1H, d, J = 3.9 Hz, H-2), 4.27 (1H, m, H-3), 4.20 (1H, m, H-4), 3.36 - 2.92 (2H, ABX, 2 x H-5), 2.74 - 2.70 (1H, septet, NCH), 1.95 - 1.63 (8H, m, cyclopentylidene protons), 1.04 - 1.03 (6H, dd, $\mathbf{Me}_2$CH); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.06 (OCO), 104.82 (C-1), 86.14 (C-2), 78.30 (C-3), 77.07 (C-4), 48.64 (NCH), 45.90 (C-5), 36.85, 36.32 ($\mathbf{C}H_2\mathbf{C}CH_2$), 23.51, 22.84 ($\mathbf{C}H_2CH_2\mathbf{C}CH_2\mathbf{C}H_2$), 22.64

(**CH$_3$**CHN), 22.34 (**CH$_3$**CHN); HRMS (EI) m/e calcd for $C_{13}H_{23}NO_4$ [M$^+$] 257.1627, found 257.1630.

1,2:3,5-di-O-cyclopentylidene-α-L-xylofuranose (55b).

Using the same procedure as described for the preparation of 1,2:3,5-di-O-cyclopentylidene-α-D-xylofuranose **55a**, 1,2:3,5-di-O-cyclopentylidene-α-L-xylofuranose **55b** was obtained with the yield of 62%: m.p. 82-84 ^{0}C; ^{1}H NMR(500 MHz, $CDCl_3$) δ 5.96 (d, J = 3.9 Hz, 1H, H-1), 4.42 (d, J = 3.9 Hz, 1H, H-2), 4.21 (d, J = 2.0 Hz, 1H, H-3), 4.09 - 3.98 (m, 3H, H-4, 2 x H-5), 1.97-1.57 (m, 16H, cyclopentylidene protons); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.20 (C-1OCOC-2), 109.20 (C-3OCOC-5), 105.05 (C-1), 84.46 (C-2), 74.22 (C-4), 71.80 (C-3), 61.47 (C-5), 39.49, 36.80, 36.16, 29.71, 24.07, 23.47, 22.77, 22.34 (cyclopentylidene carbons); MS (CI) m/e 283 (M+H$^+$).

1,2-O-cyclopentylidene-L-xylofuranose (56b).

Using the same procedure as described for the preparation of 1,2-O-cyclopentylidene-D-xylofuranose **56a**, 1,2-O-cyclopentylidene-L-xylofuranose **56b** was obtained in quantitative yield: m.p. 54-56 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (d, J = 3.9 Hz, 1H, H-1), 4.42 (d, J = 3.9 Hz, 1H, H-2), 4.30 (d, J = 2.9 Hz, 1H, H-3), 4.17 - 4.14 (m, 1H, H-4), 4.07 - 3.97 (m, 2H, 2 x H-5), 1.96 - 1.60 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.42 (OCO), 104.52 (C-1), 85.56 (C-2), 78.81 (C-3), 76.77 (C-4), 61.01 (C-5), 36.84, 36.19 (**CH_2CCH_2**), 23.47, 22.79 (**CH_2CH_2CCH_2CH_2**); MS (CI) m/e 217 (M+H$^+$).

1,2-O-cyclopentylidene-5-O-tosyl-α-L-xylofuranose (57b).

Using the same procedure as described for the preparation of 1,2-O-cyclopentylidene-5-O-tosyl-α-D-xylofuranose **57a**, 1,2-O-cyclopentylidene-5-O-tosyl-α-L-xylofuranose **57b** was obtained in quantitative yield: m.p. 95-97 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 - 7.32 (AA'BB', 4H, Ph), 5.83 (d, J = 3.9 Hz, 1H, H-1), 4.42 (d, J = 3.9 Hz, 1H, H-2), 4.42 - 4.24 (m, 3H, H-3, 2 x H-5), 4.16 - 4.08 (m, 1H, H-4), 2.42 (s, 3H, CH_3), 1.90 - 1.63 (m, 8H, cyclopentylidene protons); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 145.25 , 130.26, 130.09, 130.01, 129.96 (aromatic), 121.71 (OCO), 104.69 (C-1), 85.04 (C-2), 77.67 (C-4), 74.25 (C-3), 66.39 (C-5), 36.89, 36.30 ($\mathbf{C}H_2\mathbf{C}CH_2$), 23.42, 22.85 ($CH_2\mathbf{C}H_2\mathbf{C}CH_2\mathbf{C}H_2$), 21.63 ($CH_3$); MS (CI) m/e 371 ($M+H^+$).

1,2-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-L-xylofuranose (52).

Using the same procedure as described for the preparation of **51**, **52** was obtained in a 72% yield from 1,2-O-cyclopentylidene-5-O-tosyl-α-L-xylofuranose **57b**: m.p. 39 - 41 ^{0}C; $[\alpha]^{20}_{D}$ = -31.37 (C = 2, $CHCl_3$); ^{1}H NMR (500 MHz, CDCl3) δ 8.0 (b, NH), 5.90 (1H, d, J = 3.9 Hz, H-1), 4.38 (1H, d, J = 3.9 Hz, H-2), 4.27 (1H, m, H-3), 4.20 (1H, m, H-4), 3.36 - 2.92 (2H, ABX, 2 x H-5), 2.74 - 2.70 (1H, septet, NCH), 1.94 - 1.62 (8H, m, cyclopentylidene protons), 1.03 - 1.01 (6H, dd, J = 2.4 Hz, J = 6.4 Hz, $\mathbf{Me}_2$CH); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.01 (OCO), 104.79 (C-1), 86.10 (C-2), 78.26 (C-3), 77.04 (C-4), 48.62 (NCH), 45.87 (C-5), 36.81, 36.28 ($\mathbf{C}H_2\mathbf{C}CH_2$), 23.49, 22.81 ($CH_2\mathbf{C}H_2\mathbf{C}CH_2\mathbf{C}H_2$), 22.62 ($\mathbf{C}H_3$CHN), 22.32 ($\mathbf{C}H_3$CHN); HRMS (EI) m/e calcd for $C_{13}H_{23}NO_4$ [M^+] 257.16270, found 257.16250.

2.5.3. Experimental for Section 2.3.2.

5'-O-(*tert*-butyldimethylsilyl) thymidine (T_3,OH).

To a solution of thymidine (2.42 g, 10 mmol) in 15 ml DMF was added imidazole (1.7 g, 25 mmol) and *tert*-butyldimethylsilylchloride (1.6 g, 10.6 mmol). The solution was stirred at room temperature for 3 h. DMF was then removed in *vacuo* and the residue was dissolved in 150 ml ethyl acetate. The solution was then washed with water and the organic layer was dried over $MgSO_4$. After removing the solvent, the solid was recrystallized with ethyl acetate/pentane to afford pure 5'-O-(*tert*-butyldimethylsilyl) thymidine **6** (2.5 g, 70% yield): m.p. 193 - 194 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.0 (s, 1H, NH), 7.50 (s, 1H, H-6), 6.36 (dd, J = 5.8, 8.1 Hz, 1H, H-1'), 4.44 (m, 1H, H-3'), 4.03 (m, 1H, H-4'), 3.85 (m, 2H, H-5'), 2.66 (d, J = 3.8 Hz, 1H, OH), 2.35 (m, 1H, H-2'), 2.07 (m, 1H, H-2'), 1.89 (s, 3H, C=CMe), 0.89 (s, 9H, CMe_3), 0.09 (s, 6H, $SiMe_2$); ^{13}C NMR (125.7MHz, $CDCl_3$) δ 163.8 (C-4), 150.4 (C-2), 135.4 (C-6), 110.9 (C-5), 87.2 (C-4'), 85.0 (C-1'), 72.6 (C-3'), 63.6 (C-5'), 41.1 (C-2'), 25.9 ($SiCMe_3$), 18.3 ($SiCMe_3$), 12.5 (C=CMe), -5.4 ($SiMe_2$), -5.5 ($SiMe_2$).

5'-O-(4,4'-dimethoxytrityl) thymidine.

Triethylamine (10 ml) in 200 ml THF was injected into a solid mixture of thymidine (6.8 g, 28.0 mmol) and 4,4'-dimethoxytrityl chloride (10.2 g, 28.6 mmol) under nitrogen with stirring. The solution was stirred at room temperature for 2 h. After completion of the reaction, 10 ml of methanol was added to consume the excess DMTrCl. The mixture was stirred for 5 min and the solvent was removed by rotary evaporation. The residue was dissolved in 250 ml of ethyl acetate and the solution was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. The solid was recrystallized from ethyl acetate/hexane to obtain 5'-protected thymidine (13.0 g, 85.6%): m.p. 124 - 126 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.97 (s, 1H, NH), 7.60 (m, 1H, H-6), 6.72 - 7.42 (m, 13H, Ph), 6.42 (m, 1H, H-1'),

4.56 (m, 1H, H-3'), 4.05 (m, 1H, H-4'), 3.78 (s, 6H, OMe_2), 3.41 (m, 2H, H-5'), 2.62 (m, 1H, OH), 2.46 (m, 2H, H-2'), 1.46 (s, 3H, Me); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.0 (C-4), 157.6, 149.8 (C-2), 143.5, 135.0 (C-6), 134.7, 134.6, 129.4, 127.4, 127.3, 126.5, 112.8, 110.9 (C-5), 86.7 (C-4'), 86.2, 84.7 (C-1'), 72.5 (C-3'), 63.8 (C-5'), 55.5 (OCH_3), 41.3 (C-2'), 12.5 (CH_3).

5'-O-(4,4'-dimethoxytrityl)-3'-O-(*tert*-butyldimethlsilyl)thymidine.

To a solution of 5'-O-(4,4'-dimethoxytrityl)thymidine (13.0 g, 23.9 mmol) in 50 ml DMF was added imidazole (3.0 g, 44 mmol) and *tert*-butyl-dimethylsilyl chloride (3.6 g, 23.9 mmol). The solution was stirred at room temperature for 3 h. DMF was then removed *in vacuo* and the residue was dissolved in 300 ml ethyl acetate. The solution was washed with water and the organic layer was dried over $MgSO_4$. After concentration of the solution and recrystallization from ethyl acetate/hexane, the solid product 5'-O-(4,4'-dimethoxy)-3'-O-(*tert*-butyldimethylsilyl)thymidine was used directly for the next reaction.

3'-O-(*tert*-butyldimethylsilyl)thymidine (T_s,OH).

A solution of 5'-O-(4,4'-dimethoxytriryl)-3'-O-(*tert*-butyldimethylsilyl) thymidine (5 g, 7.6 mmol) in 100 ml 80% aqueous. acetic acid was stirred until the removal of dimethoxytrityl group from oxygen was completed. Saturated Na_2CO_3 was then added to adjust the pH of the solution to 6 - 7. The solution was extracted with ethyl acetate, the extract was dried and the mixture was chromatographed on a silica gel column (CH_2Cl_2:MeOH = 20:1) to give 3'-O-(*tert*-butyldimethylsilyl) thymidine (2.5 g, 92.6%): m.p. 93 - 95 °C (lit. 83 - 84 °C); ^1H NMR (200 MHz, $CDCl_3$) δ 9.18 (s, 1H, NH),7.36 (b, 1H, H-6), 6.12 (t, J = 6.8 Hz, 1H, H-1'), 4.44 - 4.48 (m, 1H, H-3'), 3.69 - 3.91 (m, 3H, H-4', H-5'), 2.87 (m, 1H, OH), 2.15-2.35 (m, 2H, H-2'), 1.87 (s, 3H, C=CMe), 0.86 (s, 9H, CMe_3), 0.05 (s, 6H, $SiMe_2$); ^{13}C NMR (125.7MHz, $CDCl_3$) δ 163.9 (C-4), 150.4 (C-2), 137.1 (C-6), 110.9 (C-5), 87.6 (C-4'), 86.8 (C-1'), 71.5 (C-3'), 61.9 (C-

5'), 40.4 (C-2'), 25.7 ($SiCMe_3$), 17.9 ($SiCMe_3$), 12.5 (C=CMe), -4.7 ($SiMe_2$), -4.9 ($SiMe_2$).

2-Bromo-4,5-dicyanoimidazole (46).

To a mixture of 4,5-dicyanoimidazole (1.18 g 10 mmol) and 25 ml of 0.1 M NaOH was added Br_2 (1.8 ml, 35 mmol). The mixture was stirred overnight at room temperature and then acidified with dilute HCl. The solid was filtered, rinsed with water and recrystallized from water to give dicyanobromoimidazole **46** (1.5 g, yield 76.4%): m.p. 147 - 149 °C (lit. 141 - 143°C); R_f = 0.65 (ethyl acetate:methanol = 4:1); MS(EI) 198 ([M+2], 96%), 196 ([M^+], 100%),171 (28.5), 169 (29.2), 117 (27.4), 91 (19.0), 64 (20.6), 53 (22.4), 38 (18.8).

Cyclic Phosphorochloridite (58).

To a solution of freshly distilled phosphorus trichloride (96 ml, 1,1 mmol) in 2.5 ml of anhydrous chloroform stirred at 0 °C under argon was added a mixture of **51** (257 mg, 1.0 mmol) and triethylamine (278 ml, 2.2 mmole) in 3.5 ml of anhydrous $CHCl_3$. The mixture was heated at 50 °C and the reaction was followed by ^{31}P NMR until a single peak was obtained. The product **58** was not isolated and was directly used in the following step: ^1H NMR (500 MHz, $CDCl_3$) δ 5.75 (1H, d, J = 3.9 Hz, H-1), 4.51 (1H, m, H-3), 4.37 (1H, d, J = 3.9 Hz, H-2), 4.19 - 4.17 (1H, m, H-4), 3.42 - 3.34 (1H, septet, NCH), 3.33 - 3.00 (2H, **ABX**, 2 x H-5), 1.81 - 1.51 (8H, m, cyclopentylidene protons), 1.05 (6H, d, J = 6.8 Hz, $\mathbf{Me_2}$CH); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 121.48 (OCO), 104.26 (C-1), 83.85 (d, J = 3.7 Hz, C-2), 73.01 (d, J = 6.4 Hz, C-3), 72.35 (C-4), 50.28 (d, J = 35.7 Hz, C-5), 36.93 (d, J = 5.5 Hz, NCH), 36.62, 35.91 ($\mathbf{CH_2}CCH_2$), 23.22, 22.50 ($CH_2\mathbf{CH_2}CCH_2\mathbf{CH_2}$), 20.84 (d, J = 12.8 Hz, $\mathbf{CH_3}$CHN), 19.62 (d, J = 5.5 Hz, $\mathbf{CH_3}$CHN); ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.42.

Oxazaphosphorinane (60).

The solution of **58** was cooled down to 0 °C under argon; then a solution of $\mathbf{T_3OH}$ (356 mg, 1.0 mmole) in 4.5 ml of anhydrous $CHCl_3$ and triethylamine (140 ml, 1.1 mmol) was added slowly. The mixture was heated to 50 °C until a single peak corresponding to a new product was found in the ^{31}P NMR spectrum. The solution was taken up in ethyl acetate (prewashed with a saturated solution of $NaHCO_3$), washed with saturated $NaHCO_3$ and dried over $MgSO_4$. The crude product was flash chromatographed on a silica gel column (hexane/ethyl acetate/triethylamine = 5/3/2) to furnish **60** as a white foam (510 mg, 80%): $[\alpha]^{20}_D$ = +62.91 (C = 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.19 (1H, b, NH), 7.47 (1H, d, $J_{H6\text{-}CH3C=C}$ = 1.4 Hz, H-6), 6.34 - 6.31 (1H, dd, $J_{H1'\text{-}H2a'}$ = 5.6 Hz, $J_{H1'\text{-}H2b'}$ = 8.6 Hz, H-1'), 5.89 (1H, d, $J_{H1''\text{-}H2''}$ = 3.9 Hz, H-1''), 4.57 - 4.54 (1H, ddt, $J_{H3'\text{-}H4'}$ = $J_{H3'\text{-}H2a'}$ = 2.2 Hz, $J_{H3'\text{-}H2b'}$ = 6.6 Hz, $J_{H3'\text{-}P}$ = 10.5 Hz, H-3'), 4.41 (1H, d, $J_{H1''\text{-}H2''}$ = 3.9 Hz, H-2''), 4.35 (1H, dd, $J_{H3''\text{-}H4''}$ = 2.4 Hz, $J_{H3''\text{-}P}$ = 1.0 Hz, H-3''), 4.17 (1H, q, $J_{H4''\text{-}H3''}$ = $J_{H4''\text{-}H5a''}$ = $J_{H4''\text{-}H5e''}$ = 2.4 Hz, H-4''), 4.06 (1H, q, $J_{H4'\text{-}H3'}$ = $J_{H4'\text{-}H5a'}$ = $J_{H4'\text{-}H5b'}$ = 2.2 Hz, H-4'), 3.90-3.77 (2H, ABX, 2 x H-5'), 3.47 - 3.41 (1H, d septet, $J_{NCH\text{-}CH3}$ = 6.6 Hz, $J_{NCH\text{-}P}$ = 11.7 Hz, NCH), 3.47 - 3.44 (1H, dt, $J_{H5a''\text{-}H5e''}$ = 14.2 Hz, $J_{H5a''\text{-}H4''}$ = $J_{H5a''\text{-}P}$ = 2.4 Hz, H-5a''), 3.06 - 3.01 (1H, ddd, $J_{H5e''\text{-}H4''}$ = 2.4 Hz, $J_{H5e''\text{-}H5a''}$ = 14.2 Hz, $J_{H5e''\text{-}P}$ = 7.1 Hz, H-5e''), 2.39 - 2.35 (1H, ddd, $J_{H2a'\text{-}H1'}$ = 5.9 Hz, $J_{H2a'\text{-}H3'}$ = 2.2 Hz, $J_{H2a'\text{-}H2b'}$ = 13.7 Hz, H-2'), 2.11 - 2.06 (1H, ddd, $J_{H2b'\text{-}H1'}$ = 8.4 Hz, $J_{H2b'\text{-}H3'}$ = 6.6 Hz, $J_{H2b'\text{-}H2a'}$ = 14 Hz, H-2'), 1.90 (3H, d, J = 1.5 Hz, MeC=C), 1.97 - 1.62 (8H, m, cyclopentylidene protons), 1.13 - 1.10 (6H, dd, J = 6.8 Hz, J = 7.8 Hz, $\mathbf{Me_2}$CH), 0.91 (9H, s, *t*-BuSi), 0.10 (6H, d, J = 1.5 Hz, $\mathbf{Me_2}$Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.38 (C-4), 150.10 (C-2), 135.24 (C-6), 121.46 (OCO), 110.94 (C-5), 104.63 (C-1''), 86.44 (d, J = 2.8 Hz, C-4'), 84.83 (C-1'), 84.82 (d, J = 7.3 Hz, C-2''), 73.75 (d, J = 19.2 Hz, C-3'), 73.07 (d, J = 1.8 Hz, C-4''), 71.85 (d, J = 3.7 Hz, C-3''), 63.21 (C-5'), 50.0 (d, J = 36.6 Hz, NCH), 40.28 (d, J = 5.5 Hz, C-2'), 36.92, 36.24 (CH_2CCH_2), 36.12 (d, J = 3.2 Hz, C-5''), 25.94

(SiC**C**H_3), 23.49, 22.80 (**C**H_2CH_2C**C**H_2CH_2), 22.03 (d, J = 9.2 Hz, **C**H_3CHN), 21.70 (d, J = 6.4 Hz, **C**H_3CHN), 18.36 (SiC**C**H_3), 12.52 (**C**H_3C=C), -5.37, -5.44 (**Me**$_2$Si); ^{31}P NMR (202 MHz, $CDCl_3$) δ 129.80; HRMS (FAB, glycerol) m/e calcd for $C_{29}H_{49}N_3O_9PSi$ [MH^+] 642.2973, found 642.2976.

Oxazaphosphorinane (59).

The reaction procedure and workup were analogous to those of **60** except the reaction was done at -78 °C and workup was done at 0 °C. After purification with silica gel, a mixture of **59** and **60** was obtained in a ratio of 2:3. The NMR spectrum of **59** was obtained by substracting the spectrum of **60** from the spectrum of the mixture of **59** and **60**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (1H, b, NH), 7.45 (1H, d, J = 1.4 Hz, H-6), 6.34 (1H, dd, $J_{H1'-H2a'}$ = 5.1 Hz, $J_{H1'-H2b'}$ = 8.3 Hz, H-1'), 5.97 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-1''), 4.71, 4.68 (1H, ddt, $J_{H3'-4'} = J_{H3'-2a'}$ = 2.2 Hz, $J_{H3'-2b'}$ = 6.1 Hz, $J_{H3'-P}$ = 9.0 Hz, H-3'), 4.56 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-2''), 4.09 (1H, dd, $J_{H3''-H4''}$ = 3.4 Hz, $J_{3''-P}$ = 3.2 Hz, H-3''), 4.51 (1H, dt, $J_{H4''-H3''}$ = 3.9 Hz, $J_{H4''-H5a''} = J_{H4''-H5e''}$ = 7.3 Hz, H-4''), 4.04 (1H, q, $J_{H4'-H3'} = J_{H4'-H5a'} = J_{H4'-H5b'}$ = 2.2 Hz, H-4'), 3.86-3.73 (2H, ABX, 2 x H-5'), 3.50 (1H, d septet, $J_{NCH-CH3}$ = 6.8 Hz, J_{NCH-P} = 10.7 Hz, NCH), 3.21 (1H, ddd, $J_{H5e''-H4''}$ = 7.8 Hz, $J_{H5e''-H5b''}$ = 13.7 Hz, $J_{H5e''-P}$ = 6.1 Hz, H-5e''), 3.08 (1H, ddd, $J_{H5a''-H4''}$ = 6.9 Hz, $J_{H5a''-H5e''}$ = 13.7 Hz, $J_{H5a'-P}$ = 4.7 Hz, H-5a''), 2.37 (1H, m, H-2'), 2.02 (1H, m, H-2'), 1.88 (3H, d, J = 1.5 Hz, **Me**C=C), 1.97-1.62 (8H, m, cyclopentylidene protons), 1.13-1.10 (6H, m, **Me**$_2$CH), 0.89 (9H, s, t-BuSi), 0.08 (6H, s, **Me**$_2$Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.57 (C-4), 150.08 (C-2), 135.33 (C-6), 121.42 (OCO), 110.78 (C-5), 105.76 (C-1''), 86.78 (d, J = 3.7 Hz, C-4'), 84.80 (C-1'), 84.53 (d, J = 4.6 Hz, C-2''), 73.03 (d, J = 10 Hz, C-3'), 78.72 (d, J = 2.7 Hz, C-4''), 75.82 (d, J = 2.0 Hz, C-3''), 63.06 (C-5'), 48.16 (d, J = 37.5 Hz, NCH), 40.30 (d, J = 4.5 Hz, C-2'), 37.03, 36.62 (**C**H_2C**C**H_2), 38.91 (d, J = 3.7 Hz, C-5''), 25.90 (SiC**Me**$_3$), 23.39, 23.09 (**C**H_2CH_2C**C**H_2**C**H_2), 22.58 (d, J = 8.0

Hz, CH_3CHN), 22.29 (d, J = 3.7 Hz, CH_3CHN), 18.33 ($SiCMe_3$), 12.51 ($CH_3C=C$), -5.35, -5.47 (Me_2Si); ^{31}P NMR (202 MHz, $CDCl_3$) δ 138.70.

Phosphorothioates (62/64).

A mixture of phosphoramidite **60** (15 mg, 0.0234 mmole), **T_5-OH** (10 mg, 1.2 eq) and 2-bromo-4,5-dicyanoimidazole **46** (9.2 mg, 2.0 eq) was dried in high vacuo overnight. Anhydrous acetonitrile (0.6 ml) was added at 0 °C under argon. The solid dissolved instantly. The reaction was warmed to RT and its course was followed by ^{31}P NMR. Within 5 minutes, the peak corresponding to the phosphoramidite had disappeared and two new peaks corresponding to **61** and **63** (143.76 ppm : 142.55 ppm = 6:1) appeared: MS (FAB, nitrobenzyl alcohol) $[M + H]^+$ calcd for $C_{45}H_{77}N_5PO_{14}Si_2$ phosphite triester 998, found 998.4. This product was used in the following sulfurization without isolation. To this solution was added Beaucage's reagent (5.6 mg, 1.2 eq.) in 140 µl of acetonitrile. Instantaneously the ^{31}P NMR showed another two peaks corresponding to **62** and **64** (68.23 ppm : 68.43 ppm = 6:1), while the peaks corresponding to the starting material disappeared. The solvent was evaporated and the mixture was redissolved in ethyl acetate, washed with saturated $NaHCO_3$ and water and dried over $MgSO_4$. The crude product was purified on a silica gel column (ethyl acetate:methanol = 95:5) to give a white solid **62/64** (22 mg, 91%). When a similar reaction was carried out at 0 °C for 4 h and at -15 °C for 6 h, using chloroform as the solvent, the ratio of **62** and **64** increased to 40:1 and 68:1, respectively. Chromatography of the reaction mixture obtained at -15 °C only provided one isomer **62**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (1H, d, J = 1.5 Hz, ^{5}H-6), 7.23 (1H, d, J = 1.5 Hz, ^{3}H-6), 6.37 - 6.35 (1H, dd, J = 5.4 Hz, J = 9.3 Hz, ^{5}H-1'), 6,18 (1H, t, J = 6.4 Hz, ^{3}H-1'), 5.87 (1H, d, J = 3.9 Hz, H-1''), 5.14 (1H, dd, J = 5.4 Hz, J = 9.3 Hz, ^{5}H-3'), 4.84 (1H, dd, J = 2.9 Hz, J = 12.2 Hz, H-3''), 4.59 (1H, d, J = 3.9 Hz, H-2''),

4.35 (m, 1H, H-4"), 4.26 - 4.20 (4H, m, ^{3}H-3', 2 x ^{3}H-5', ^{5}H-4'), 4.00 (1H, m, ^{3}H-4'), 3.86 (2H, m, 2 x ^{5}H-5'), 2.83 (2H, m, 2 x H-5"), 2.77 (1H, septet, NCH), 2.45 (1H, m, ^{5}H-2'), 2.25 (2H, m, 2 x ^{3}H-2'), 2.15 (1H, m, ^{5}H-2'), 1.92 (3H, d, J = 1.0 Hz, $^{3}CH_3C$=C), 1.90 (3H, d, J = 1.0 Hz, $^{5}CH_3C$=C), 1.97-1.61 (8H, m, cyclopentylidene protons), 1.02 (6H, dd, J = 5.9 Hz, Me_2CHN), 0.92 (9H, s, ^{3}t-BuSi), 0.87 (9H, s, ^{5}t-BuSi), 0.13 (6H, s, Me_2Si), 0.07 (6H, s, Me_2Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.97, 163.66 (^{5}C-4, ^{3}C-4), 150.39, 150.04 (^{5}C-2, ^{3}C-2), 135.58, 134.49 (^{5}C-6, ^{3}C-6), 121.77 (OCO), 111.26, 111.04 (^{5}C-5, ^{3}C-5), 104.00 (C-1"), 85.57 (d, J = 6.4 Hz, ^{5}C-4'), 85.48 (^{3}C-1'), 84.65 (d, J = 9.2 Hz, ^{3}C-4'), 84.39 (^{5}C-1'), 83.47 (C-2"), 80.70 (d, J = 4.6 Hz, C-3"), 80.68 (d, J = 4.6 Hz, ^{5}C-3'), 79.00 (d, J = 7.3 Hz, ^{3}C-3'), 71.49 (C-4"), 67.66, 67.52 (d, J = 4.6 Hz, ^{3}C-5'), 63.47 (^{5}C-5'), 48.93 (NCH), 45.23 (C-5"), 40.37 (^{3}C-2'), 39.12 (d, J = 6.4 Hz, ^{5}C-2'), 37.06, 36.16 ($\mathbf{CH_2CCH_2}$), 25.88, 25.60 ($^{5}SiCMe_3$, $^{3}SiCMe_3$), 23.52, 22.85 ($\mathbf{CH_2CH_2CCH_2CH_2}$), 22.65 22.39 ($NCHMe_2$), 18.28, 17.81 ($^{5}SiCMe_3$, $^{3}SiCMe_3$), 12.51, 12.46 (^{5}C=C$\mathbf{CH_3}$, ^{3}C=C$\mathbf{CH_3}$), -4.66, -4.83, -5.40, -5.46 ($^{5}SiMe_2$, $^{3}SiMe_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 68.23; HRMS (FAB, glycerol-CsI) m/e calcd for $C_{45}H_{77}N_5O_{14}PSSi_2$ [MH^+] 1030.4460, found 1030.4464.

Dithymidine Phosphorothioate (65).

The protected phosphorothioate dinucleoside **62** (14 mg, 0.0136 mmol) was dissolved in 1.0 ml of 70% TFA in H_2O at 0 ^{0}C and allowed to stir at RT for 2 h. Evaporation of the solvent and coevaporation with methanol to furnish a white solid which was purified by chromatography to give a white solid **65** (7.2 mg, 94%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.87 (s, 1H, ^{5}H-6), 7.85 (1H, s, ^{3}H-6), 6.36 - 6.33 (1H, dd, J = 6.4 Hz, J = 7.8 Hz, ^{5}H-

1'), 6.30 - 6.27 (1H, dd, J = 6.4 Hz, J = 7.3 Hz, ^{3}H-1'), 5.06 - 5.03 (1H, m, ^{5}H-3'), 4.53 - 5.52 (1H, m, ^{3}H-3'), 4.21 - 4.06 (4H, m, ^{5}H-4', 2 x ^{3}H-5', ^{3}H-4'), 3.84 - 3.80 (2H, m, 2 x ^{5}H-5'), 2.50 - 2.46 (1H, m, ^{5}H-2'), 2.31 - 2.24 (2H, m, 2x^{3}H-2'), 2.21 - 2.17 (1H, m, ^{5}H-2'), 1.96 (3H, s, $^{3}CH_3C$=C), 1.87 (3H, s, $^{5}CH_3C$=C); ^{31}P NMR (202 MHz, D_2O) δ 55.70; HRMS (FAB, glycerol) m/e calcd for $C_{20}H_{28}N_4O_{11}PS$ [MH^+] 563.11347, found 563.11350. HPLC showed only a single peak. When the mixture of protected phosphorothioates **62/64** was hydrolyzed in the same reaction condition, the dithymidine phosphorothioates **65/66** were obtained with an Sp : Rp diastereomer ratio the same as the ratio of **62** to **64**.

Cyclic Phosphorochloridite (67).

Obtained by the procedure described for the preparation of **58**. The product was not isolated and it was used directly in the following step. **67**: ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.75.

Oxazaphosphoranine (68).

Obtained from **67** by the procedure described for the preparation of **60**: m.p. 99 - 101 ^{0}C; $[\alpha]^{20}_{D}$ = -72.00 (c = 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (b, 1H, NH), 7.46 (s, 1H, H-6), 6.33 - 6.30 (dd, J = 5.9 Hz, J = 7.8 Hz, 1H, H-1'), 5.88 (d, J = 3.9 Hz, 1H, H-1''), 4.56 - 4.53 (m, 1H, H-3'), 4.43 (d, J = 3.9 Hz, 1H, H-2''), 4.35 (m, 1H, H-3''), 4.18 (q, J = 2.0 Hz, 1H, H-4''), 4.05 (m, 1H, H-4'), 3.90 - 3.76 (ABX, 2H, 2 x H-5'), 3.45 - 3.42 (m, 2H, H-5'', NCH), 3.03 - 2.99 (m, 1H, H-5''), 2.38 - 2.35 (m, 1H, H-2'), 2.12 - 2.06 (m, 1H, H-2'), 1.89 (s, 3H, MeC=C), 1.96 - 1.62 (m, 8H, cyclopentylidene protons), 1.11 - 1.08 (dd, 6H, J = 6.4 Hz, J = 11.2 Hz, $\mathbf{Me_2}$CH), 0.90 (s, 9H, t-BuSi), 0.09 (d, J = 2.0 Hz, 6H, Me_2Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ

163.74 (C-4), 150.31 (C-2), 135.18 (C-6), 121.44 (OCO), 110.93 (C-5), 104.59 (C-1''), 86.59 (d, J = 5.5 Hz, C-4'), 84.74 (d, J = 2.8 Hz, C-2''), 84.70 (C-1'), 73.96 (d, J = 17.4 Hz, C-3'), 73.06 (d, J = 1.8 Hz, C-4''), 71.80 (d, J = 4.6 Hz, C-3''), 62.98 (C-5'), 49.91 (d, J = 36.6 Hz, NCH), 39.93 (d, J = 2.8 Hz, C-2'), 36.86, 36.19 ($\mathbf{CH_2}CCH_2$), 36.09 (d, J = 3.1 Hz, C-5''), 25.92 ($SiC\mathbf{Me_3}$), 23.45, 22.76 ($\mathbf{CH_2}CH_2CCH_2CH_2$), 21.95 (d, J = 9.2 Hz, $\mathbf{CH_3}CHN$), 21.66 (d, J = 6.4 Hz, $\mathbf{CH_3}CHN$), 18.35 ($SiCMe_3$), 12.52 ($\mathbf{CH_3}C=C$), -5.39, -5.45 ($\mathbf{Me_2}Si$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 129.34; HRMS (FAB, glycerol) m/e calcd for $C_{29}H_{49}N_3O_9PSi$ [MH^+] 642.2973, found 642.2976.

Phosphorothioates (70/72).

A mixture of **68** (15 mg, 0.0234 mmol), **T_sOH** (10 mg, 1.2 eq.) and 4,5-dicyano-2-bromo-imidazole **46** (9.17 mg, 2.0 eq.) was dried under high vacuum overnight. Anhydrous acetonitrile (0.6 ml) was added at 0 °C under Ar. The solid was dissolved instantly. The reaction was followed by ^{31}P NMR. Within 5 minutes, the peak corresponding to the phosphoramidite at 129.34 ppm was replaced by two new peaks corresponding to phosphite triesters **69** and **71** (142.14 ppm:140.67 ppm = 1:7). To this solution was added Beaucage's reagent (5.6 mg, 1.2 eq.) in 140 μl of acetonitrile . Instantaneously the ^{31}P NMR showed another two peaks corresponding to **70** and **72** (67.44 ppm : 68.60 ppm = 1 : 7). The work-up and purification step was the same as that of **62/64** to give a white solid **70/72** (23 mg, 91%). When a similar reaction was carried out at -15 °C for 6 h, using chloroform as the solvent, the ratio of **70** and **72** increased to 1:70. After purification, only one product **72** was obtained: ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, J = 1.5 Hz, 1H, ^{5}H-6), 7.27 (d, J = 1.5 Hz, 1H, ^{3}H-6), 6.34 - 6.31 (dd, 1H, J = 5.2 Hz, J = 9.3 Hz, ^{5}H-1'), 6.12 - 6.09 (t, 1H, J = 6.6 Hz, ^{3}H-1'), 5.86 (d, J = 3.4 Hz, 1H, H-1''), 5.16 (dd, 1H, J = 5.6 Hz, J = 9.8 Hz, ^{5}H-3'), 4.82 (dd, J = 2.7 Hz, J = 11.7

Hz, 1H, H-3''), 4.55 (d, J = 3.9 Hz, 1H, H-2''), 4.39 - 4.37 (m, 2H, ^{3}H-3', H-4''), 4.25 - 4.21 (m, 3H, 2 x ^{3}H-5', ^{5}H-4'), 3.98 (m, 1H, ^{3}H-4'), 3.91 - 3.85 (m, 2H, 2 x ^{5}H-5'), 2.84 (d, J = 6.4 Hz, 2H, 2 x H-5''), 2.81 (m, 1H, NCH), 2.52 - 2.47 (m, 1H, ^{5}H-2'), 2.25 - 2.23 (m, 2H, 2 x ^{3}H-2'), 2.08 - 2.02 (m, 1H, ^{5}H-2'), 1.91 (d, 3H, J = 1.0 Hz, $^{3}CH_3C$=C), 1.89 (d, 3H, J = 1.0 Hz, $^{5}CH_3C$=C), 1.92-1.65 (m, 8H, cyclopentylidene protons), 1.05 (d, J = 5.9 Hz, 6H, Me_2CHN), 0.90 (s, 9H, ^{3}t-BuSi), 0.86 (s, 9H, ^{5}t-BuSi), 0.11 (s, 6H, Me_2Si), 0.05 (s, 6H, Me_2Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.80, 163.59 (^{5}C-4, ^{3}C-4), 150.33, 150.15 (^{5}C-2, ^{3}C-2), 136.13, 134.64 (^{5}C-6, ^{3}C-6), 122.03 (OCO), 111.43, 111.11 (^{5}C-5, ^{3}C-5), 104.28 (C-1''), 86.29 (^{3}C-1'), 85.93 (d, J = 6.4 Hz, ^{5}C-4'), 84.78 (d, J = 10 Hz, ^{3}C-4'), 84.69 (^{5}C-1'), 83.59 (C-2''), 80.83 (d, J = 5.5 Hz, C-3''), 80.68 (d, J = 4.6 Hz, ^{5}C-3'), 79.00 (d, J = 8.2 Hz, ^{3}C-3'), 71.30 (C-4''), 67.36 (d, J = 5.5 Hz, ^{3}C-5'), 63.37 (^{5}C-5'), 48.91 (NCH), 45.18 (C-5''), 40.35 (^{3}C-2'), 39.11 (d, J = 3.7 Hz, ^{5}C-2'), 37.14, 36.23 (**CH_2CCH_2**), 25.88, 25.65 ($^{5}Si\mathbf{C}Me_3$, $^{3}Si\mathbf{C}Me_3$), 23.55, 22.89 (**$CH_2CH_2CCH_2CH_2$**), 22.80, 22.55 ($NCH\mathbf{Me}_2$), 18.28, 17.86 ($^{5}Si\mathbf{C}Me_3$, $^{3}Si\mathbf{C}Me_3$), 12.52, 12.49 (^{3}C=C**CH_3**, ^{5}C=CCH_3), -4.66, -4.83, -5.40, -5.46 ($^{5}Si\mathbf{Me}_2$, $^{3}Si\mathbf{Me}_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 68.60; HRMS (FAB, glycerol-CsI) m/e calcd for $C_{45}H_{77}N_5O_{14}PSSi_2$ [MH$^+$] 1030.4460, found 1030.4464.

Dithymidine Phosphorothioate (66).

The protected phosphorothioate **72** (15 mg, 0.014 mmol) was dissolved in 1.0 ml of 70% TFA in H_2O at 0 ^{0}C and allowed to stir at RT for 2 h. Evaporation of the solvent and coevaporation with methanol furnished a white solid which was purified by chromatography to give a white solid **66** (7.3 mg, 94%): ^{1}H NMR (500 MHz, CD_3OD) δ

7.91 (s, 1H, ^{5}H-6), 7.86 (s, 1H, ^{3}H-6), 6.36 - 6.33 (dd, J = 6.4 Hz, J = 7.8 Hz, 1H, ^{5}H-1'), 6.29 - 6.26 (dd, J = 5.9 Hz, J = 7.8 Hz, ^{3}H-1'), 5.08 - 5.05 (m, 1H, ^{5}H-3'), 4.52 - 5.51 (m, 1H, ^{3}H-3'), 4.21 (m, 1H, ^{5}H-4'), 4.14 - 4.11 (m, 2H, 2 x ^{3}H-5'), 4.04 (m, 1H, ^{3}H-4'), 3.86 - 3.79 (m, 2H, 2 x ^{5}H-5'), 2.48 - 2.44 (m, 1H, ^{3}H-2'), 2.31 - 2.24 (m, 2H, 2 x ^{5}H-2'), 2.23 - 2.16 (m, 1H, ^{3}H-2'), 1.97 (s, 3H, $^3CH_3C$=C), 1.87 (s, 3H, $^5CH_3C$=C); ^{31}P NMR (202 MHz, D_2O) δ 56.10; HRMS (FAB, glycerol) m/e calcd for $C_{20}H_{28}N_4O_{11}PS$ [MH$^+$] 563.11347, found 563.11350.

2.5.4. Experimental for Section 2.3.3.

Phosphorothioamidate (60S).

To a solution of phosphoramidite **60** (46 mg, 0.075 mmol) in dry acetonitrile (1 ml) was added Beaucage's reagent (1.2 eq) at room temperature. The reaction mixture was stirred for 5 min. After flash chromatography (ethyl acetate:hexane = 1:2), white solid phosphorothioamidate **60S** was obtained in quantitative yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (b, 1H, NH), 7.51 (d, 1H, J = 1.2 Hz, H-6), 6.36 - 6.33 (dd, $J_{H1'\text{-}H2a'}$ = 5.1 Hz, $J_{H1'\text{-}H2b'}$ = 9.3 Hz, 1H, H-1'), 5.94 (d, $J_{H1''\text{-}H2''}$ = 3.9 Hz, 1H, H-1''), 5.05 - 5.02 (dd, $J_{H3'\text{-}H2'}$ = 5.6 Hz, $J_{H3'\text{-}P}$ = 10 Hz, 1H, H-3'), 4.67 (t, $J_{H3''\text{-}H4''} = J_{H3''\text{-}P}$ = 2.4 Hz, 1H, H-3''), 4.57 (d, $J_{H2''\text{-}H1''}$ = 3.9 Hz, 1H, H-2''), 4.30 - 4.29 (pentet, $J_{H4''\text{-}H3''} = J_{H4''\text{-}H5a''} = J_{H4''\text{-}H5e''} = J_{H4''\text{-}P}$ = 2.4 Hz, 1H, H-4''), 4.27 (m, 1H, H-4'), 4.25 (septet, 1H, CHN), 3.38 (AB, 2H, 2 x H-5'), 3.46 - 3.39 (ddd, $J_{H5e''\text{-}H4''}$ = 2.7 Hz, $J_{H5e''\text{-}H5a''}$ = 14.3 Hz, $J_{H5e''\text{-}P}$ = 20 Hz, 1H, H-5e''), 3.35 - 3.30 (dt, $J_{H5a''\text{-}H5e''}$ = 14.3 Hz, $J_{H5a''\text{-}H4''} = J_{H5a''\text{-}P}$ = 3.2 Hz, 1H, H-5a''), 2.41 (m, 1H, H-2a'), 2.09 (m, 1H, H-2b'), 1.89 (d, J = 1.5 Hz, 3H, CH_3C=C), 1.96 - 1.62 (m, 8H, cyclopentylidene protons), 1.11 (d, J = 6.8 Hz, 3H, **CH_3**CHN), 1.05 (d, J = 6.8 Hz, 3H, **CH_3**CHN), 0.90 (s, 9H, t-BuSi), 0.11 (s, 6H, Me_2Si); ^{13}C NMR

(125.7 MHz, $CDCl_3$) δ 163.81 (C-4), 150.53 (C-2), 134.92 (C-6), 121.91 (OCO), 111.23 (C-5), 104.68 (C-1''), 85.91 (d, J = 1.8 Hz, C-4'), 84.80 (C-1'), 84.00 (d, J = 11.0 Hz, C-2''), 80.95 (d, J = 9.2 Hz, C-3''), 78.25 (d, J = 5.5 Hz, C-3'), 72.60 (d, J = 4.6 Hz, C-4''), 63.55 (C-5'), 47.90 (d, J = 6.4 Hz, CHN), 39.40 (d, J = 7.3 Hz, C-5''), 39.25 (C-2'), 36.85, 36.20 ($\mathbf{CH_2}CCH_2$), 25.86 ($SiCMe_3$), 23.35, 22.78 ($\mathbf{CH_2}CH_2CCH_2\mathbf{CH_2}$), 20.60 (d, J = 6.4 Hz, NCH**Me**), 20.20 (d, J = 2.7 Hz, NCH**Me**), 18.25 ($SiCMe_3$), 12.44 ($C{=}CCH_3$), -5.47, -5.50 ($SiMe_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 67.54; HRMS (FAB, glycerol) m/e calcd for $C_{29}H_{49}N_3O_9PSSi$ [MH^+] 674.2693, found 674.2696.

Phosphorothioamidate (59S).

To a solution of a mixture of phosphoramidite **59** and **60** in a ratio of 2 : 3 (45 mg, 0.075 mmol) in dry chloroform (1.0 ml) was added Beaucage's reagent (1.2 eq) at room temperature. The reaction mixture was stirred for 5 min. After flash chromatography (ethyl acetate:hexane = 1:3), the slow eluent (25 mg) is **60S** according to ^{31}P NMR (67.54 ppm), while the fast eluent (18 mg) is **59S** by ^{31}P NMR (72.35 ppm): ^1H NMR (500 MHz, $CDCl_3$) δ 8.41 (b, 1H, NH), 7.52 (d, 1H, J = 1.2 Hz, H-6), 6.36 - 6.33 (dd, $J_{H1'-H2'a}$ = 5.3 Hz, $J_{H1'-H2'b}$ = 9.3 Hz, 1H, H-1'), 6.00 (d, 1H, $J_{H1''-H2''}$ = 4 Hz, H-1''), 5.20 - 5.17 (ddd, 1H, $J_{H3'-H2'}$ = 5.6 Hz, $J_{H3'-4'}$ = 1 Hz, $J_{H3'-P}$ = 10 Hz, H-3'), 4.65 - 4.63 (dd, 1H, $J_{H3''-H4''}$ = 4.1 Hz, $J_{H3''-H2''}$ = 5.3 Hz, H-3''), 4.63 (d, 1H, $J_{H2''-H1''}$ = 4 Hz, H-2''), 4.56 - 4.52 (ddd, 1H, $J_{H4''-H3''}$ = 4.1 Hz, $J_{H4''-H5a''}$ = $J_{H4''-H5b''}$ = 6.6 Hz, H-4''), 4.16 (q, 1H, $J_{H4'-H3'}$ = $J_{H4'-H5a'}$ = $J_{H4'-H5b'}$ = 1 Hz, H-4'), 3.95 (septet, 1H, CHN), 3.88 (d, 2H, 2 x H-5'), 3.45 - 3.37 (ddd, 1H, $J_{H5a''-H4''}$ = 6.4 Hz, $J_{H5a''-H5b''}$ = 14, $J_{H5a''-P}$ = 19 Hz, H-5a''), 3.13 - 3.07 (ddd, 1H, $J_{H5b''-H4''}$ = 6.5 Hz, $J_{H5b''-H5a''}$ = 14 Hz, $J_{5b''-P}$ = 12 Hz, H-5b''), 2.49 - 2.46 (dd, 1H, $J_{H2a'-H2b'}$ = 14 Hz, $J_{H2a'-H3'}$ = 5.4 Hz, H-2a'), 2.10 - 2.04 (ddd, 1H, $J_{H2b'-H2a'}$ = 15 Hz, $J_{H2b'-H1'}$ = 9 Hz, $J_{H2b'-H3'}$ = 5.3 Hz, H-2b'), 1.89 (d, 3H, J = 1 Hz, $CH_3C{=}C$), 1.96 - 1.62

(m, 8H, cyclopentylidene protons), 1.11 (d, 3H, J = 6.8 Hz, CH_3CHN), 1.07 (d, 3H, J = 6.8 Hz, CH_3CHN), 0.91 (s, 9H, t-BuSi), 0.12 (s, 6H, Me_2Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.43 (C-4), 150.13 (C-2), 135.07 (C-6), 121.87 (OCO), 111.15 (C-5), 105.65 (C-1''), 86.25 (d, J = 6.4 Hz, C-4'), 84.72 (C-1'), 83.97 (d, J = 12 Hz, C-2''), 80.83 (d, J = 6.3 Hz, C-3''), 79.13 (d, J = 4.6 Hz, C-3'), 77.80 (d, J = 1.8 Hz, C-4''), 63.46 (C-5'), 46.90 (d, J = 6.4 Hz, CNH), 40.74 (C-2'), 39.36 (d, J = 5.5 Hz, C-5''), 37.04, 36.62 ($\mathbf{CH_2}CCH_2$), 25.93 ($SiCMe_3$), 23.37, 23.11 ($\mathbf{CH_2}CH_2CCH_2\mathbf{CH_2}$), 20.97 (d, J = 2.7 Hz, NCH**Me**), 20.57 (d, J = 4.6 Hz, NCH**Me**), 18.34 ($SiCMe_3$), 12.49 ($C=CCH_3$), -5.39 ($SiMe_2$); HRMS (FAB, glycerol) m/e calcd for $C_{29}H_{49}N_3O_9PSSi$ [MH^+] 674.2693, found 674.2696.

Synthesis of 73a-d and 74a-d:

1,2-O-isopropylidene-5'-O-tosyl-α–D-xylofuranose (76).

To a solution of 1,2-O-isopropylidene-α–D-xylofuranose **75** (11.4 g, 60 mmol) in dry pyridine (100 ml) under nitrogen atmosphere at 0 °C, *p*-toluenesulfonyl chloride (13.2 g, 1.15 eq.) was added. The reaction mixture was stirred at 0 °C for 3 h. Then it was quenched with water and the solution evaporated and coevaporated with toluene in vacuo twice. The mixture was dissolved in chloroform, washed with water three times and dried over $MgSO_4$. Removal of the solvent furnished a white solid (20.46 g, 99%) which was used for the preparation of 1,2-O-isopropylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **48** without further purification: ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.26 (AA'BB', 4H, Ph), 5.88 (d, J = 3.9 Hz, 1H, H-1), 4.51 (d, J = 3.9 Hz, 1H, H-2), 4.35 - 4.28 (m, 3H, H-3, 2 x H-5), 4.18 - 4.11 (m, 1H, H-4), 2.45 (s, 3H, OCH_3), 2.22 (d, J = 4.8 Hz, 1H, OH), 1.46 (s, 3H, CCH_3), 1.30 (s, 3H, CCH_3); MS (CI) m/e 345 ($M+H^+$).

1,2-O-isopropylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose (48).

Using the same procedure as described for the preparation of **51**, 1,2-O-isopropylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **48** was obtained in a 80% yield from 1,2-O-isopropylidene-5-O-tosyl-α-L-xylofuranose **76**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.0 (b, NH), 5.91 (1H, d, J = 3.9 Hz, H-1), 4.44 (1H, d, J = 3.9 Hz, H-2), 4.25 - 4.18 (2H, m, H-3, H-4), 3.39 - 2.90 (2H, ABX, 2 x H-5), 2.79 - 2.66 (1H, septet, NCH), 1.44 (3H, s, CCH_3), 1.03 (6H, d, J = 6.4 Hz, $\mathbf{Me_2}$CH); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.43 (OCO), 105.12 (C-1), 86.14 (C-2), 78.23 (C-3), 76.91 (C-4), 48.84 (NCH), 45.90 (C-5), 26.81, 26.17 (CH_3CCH_3), 22.64 ($\mathbf{CH_3}$CHN), 22.34 ($\mathbf{CH_3}$CHN); MS (CI) m/e 232 ([M+H$^+$], 100%).

Cyclic Phosphorochloridite (77).

Obtained by the procedure described for the preparation of **58**. The product was not isolated and it was used directly in the following step. ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.31.

Oxazaphosphorinane (73a).

Using the procedure described for the preparation of **60, 73a** was obtained from the cyclic phosphorochloridite **77** in 84% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (1H, b, NH), 7.50 (1H, d, $J_{H6\text{-}CH3C=C}$ = 1.0 Hz, H-6), 6.35 - 6.33 (1H, dd, $J_{H1'\text{-}H2a'}$ = 5.4 Hz, $J_{H1'\text{-}H2b'}$ = 8.3 Hz, H-1'), 5.94 (1H, d, $J_{H1''\text{-}H2''}$ = 3.9 Hz, H-1''), 4.60 - 4.57 (1H, m, H-3'), 4.51 (1H, d, $J_{H1''\text{-}H2''}$ = 3.9 Hz, H-2''), 4.36 (1H, m, H-3''), 4.18 (1H, m, H-4''), 4.09 (1H, m, H-4'), 3.91-3.79 (2H, ABX, 2 x H-5'), 3.49 - 3.47 (1H, m, H-5a''), 3.47 - 3.43 (1H, septet, NCH), 3.08 - 3.03 (1H, m, H-5e''), 2.41 - 2.37 (1H, m, H-2'), 2.13 - 2.07 (1H, m, H-2'), 1.92 (3H, d, J = 1.0 Hz, MeC=C), 1.49 (3H, s, $\mathbf{CH_3}CCH_3$), 1.31 (3H, s, $CH_3C\mathbf{CH_3}$), 1.14 - 1.11 (6H, m, $\mathbf{Me_2}$CH), 0.93 (9H, s, t-BuSi), 0.12 (6H, d, J

= 1.5 Hz, **Me**$_2$Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.38 (C-4), 150.50 (C-2), 135.24 (C-6), 111.61 (OCO), 110.94 (C-5), 104.78 (C-1''), 86.34 (C-4'), 84.73 (C-1'), 84.82 (C-2"), 73.64 (d, J = 18.1 Hz, C-3'), 72.32 (C-4"), 71.77 (C-3"), 63.11 (C-5'), 49.92 (d, J = 36.6 Hz, NCH), 40.24 (C-2'), 36.01 (C-5"), 26.62, 26.09 (**Me**$_2$C), 25.94 (SiC**CH**$_3$), 22.03, 21.80 (**Me**$_2$CHN), 18.27 (Si**C**CH$_3$), 12.46 (**CH**$_3$C=C), -5.37, -5.44 (**Me**$_2$Si); ^{31}P NMR (202 MHz, $CDCl_3$) δ 129.00; MS (FAB, NBA) m/e [MH$^+$] 616.

Phosphorothioamidate (74a).

Using the procedure described for the preparation of **60S**, **74a** was obtained in quantitative yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (1H, b, NH), 7.52 (1H, d, $J_{H6\text{-}CH3C=C}$ = 1.0 Hz, H-6), 6.36 - 6.33 (1H, dd, $J_{H1'\text{-}H2a'}$ = 5.1 Hz, $J_{H1'\text{-}H2b'}$ = 9.0 Hz, H-1'), 5.96 (1H, d, $J_{H1''\text{-}H2''}$ = 3.7 Hz, H-1"), 5.03 (1H, dd, J = 5.6 Hz, J = 10.0 Hz, H-3'), 4.66(1H, m, H-3''), 4.51 (1H, d, $J_{H1''\text{-}H2''}$ = 3.9 Hz, H-2"), 4.27 - 4.23 (3H, m, H-4', H-4'', NCH), 3.92 - 3.86 (2H, m, 2 x H-5'), 3.47 - 3.32 (2H, m, 2 x H-5''), 2.43 - 2.39 (1H, m, H-2'), 2.12 - 2.06 (1H, m, H-2'), 1.89 (3H, s, MeC=C), 1.47 (3H, s, **CH**$_3$CCH$_3$), 1.29 (3H, s, CH$_3$C**CH**$_3$), 1.10 (3H, d, J = 6.8 Hz, **Me**$_2$CH), 1.05 (3H, d, J = 6.8 Hz, **Me**$_2$CH), 0.90 (9H, s, t-BuSi), 0.11 (6H, s, **Me**$_2$Si); ^{13}C NMR (125.7MHz, $CDCl_3$) δ 163.65 (C-4), 150.41 (C-2), 134.97 (C-6), 112.32 (OCO), 111.25 (C-5), 104.94 (C-1''), 85.96 (d, J = 2.8 Hz, C-4'), 84.85 (C-1'), 84.12 (d, J = 11.0 Hz, C-2"), 80.95 (d, J = 9.2 Hz, C-3''), 78.29 (d, J = 5.5 Hz, C-3'), 72.45(d, J = 4.6 Hz, C-4"), 63.61 (C-5'), 48.00 (d, J = 6.4 Hz, NCH), 39.46 (d, J = 7.3 Hz, C-5"), 39.23 (C-2'), 26.64, 26.60 (**Me**$_2$C), 25.90 (SiC**CH**$_3$), 20.65(d, J = 5.5 Hz, CH$_3$CHN), 20.47 (d, J = 2.8 Hz, CH$_3$CHN), 18.30 (Si**C**CH$_3$), 12.49 (**CH**$_3$C=C), -5.42, -5.45 (**Me**$_2$Si); ^{31}P NMR (202 MHz, $CDCl_3$) δ 67.50; HRMS (FAB, glycerol) m/e calcd for $C_{27}H_{47}N_3O_9PSSi$ [MH$^+$] 648.2542, found 648.2540.

Oxazaphosphorinane (73b).

Using the procedure described for the preparation of **73a**, **73b** was obtained with yield of 60%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.93 (1H, d, J = 3.7 Hz, H-1), 4.51 (1H, d, J = 3.7 Hz, H-2), 4.41 (1H, m, H-3), 4.17 - 4.13 (2H, m, H-4, OCH), 3.49 - 3.46 (1H, m, H-5), 3.40 (1H, septet, NCH), 3.01 - 2.99 (1H, m, H-5), 1.47 (3H, s, CCH_3), 1.30 (3H, s, CCH_3), 1.23 (6H, m, OCH**Me$_2$**), 1.10 (6H, m, NCH**Me$_2$**); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.48 (OCO), 104.91 (C-1), 84.94 (d, J = 2.8 Hz, C-2), 73.48 (C-4), 71.15(d, J = 2.8 Hz, C-3), 67.14 (d, J = 18.3, OCH), 49.74 (d, J = 35.7 Hz, NCH), 36.24 (d, J = 3.7 Hz, C-5), 26.68, 26.19 (**Me$_2$**C), 24.56 (d, J = 4.6 Hz, OCH**CH$_3$**), 24.33 (d, J = 2.7 Hz, OCH**CH$_3$**), 21.91 (d, J = 10.1 Hz, NCH**CH$_3$**), 21.39 (d, J = 6.4 Hz, NCH**CH$_3$**); ^{31}P NMR (202 MHz, $CDCl_3$) δ 128.18.

Phosphorothioamidate (74b).

Using the procedure described for the preparation of **60S**, **74b** was obtained in quantitative yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, d, J = 3.7 Hz, H-1), 4.71 - 4.66 (1H, m, OCH), 4.65 (1H, d, J = 3.7 Hz, H-2), 4.60 (1H, m, H-3), 4.28 (1H, septet, NCH), 4.24 (1H, m, H-4), 3.42 - 3.32 (2H, m, 2 x H-5), 1.47 (3H, s, CCH_3), 1.31 (3H, d, J = 6.4 Hz, OCH**CH$_3$**), 1.30 (3H, s, CCH_3), 1.27 (3H, d, J = 6.1 Hz, OCH**CH$_3$**), 1.10 (3H, d, J = 6.6 Hz, NCH**CH$_3$**), 1.04 (3H, d, J = 6.4 Hz, NCH**CH$_3$**) ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 112.08 (OCO), 105.04 (C-1), 84.20 (d, J = 11.0 Hz, C-2), 80.33 (d, J = 9.1 Hz, C-3), 71.94 (d, J = 4.6 Hz, C-4), 71.87 (d, J = 6.4 Hz, OCH), 47.60 (d, J = 6.4 Hz, NCH), 39.20 (C-5), 26.68, 26.20 (**Me$_2$**C), 23.59 (d, J = 6.4 Hz, OCH**CH$_3$**), 23.40 (d, J = 3.7 Hz, OCH**CH$_3$**), 20.69 (d, J = 7.3 Hz, NCH**CH$_3$**), 20.10 (d, J = 1.8 Hz, NCH**CH$_3$**); ^{31}P NMR (202 MHz, $CDCl_3$) δ 67.08; HRMS (FAB, glycerol) m/e calcd for $C_{14}H_{27}NO_5PS$ [MH^+] 352.1349, found 352.1348.

Oxazaphosphorinane (73c).

Using the procedure described for **73a, 73c** was obtained with a yield of 78%: ^{31}P NMR (202 MHz, $CDCl_3$) δ 125.31.

Phosphorothioamidate (74c).

Using the procedure described for the preparation of **74a, 74c** was obtained in quantitative yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 - 7.34 (4H, AA'BB', aromatic protons), 6.02 (1H, d, J = 3.4 Hz, H-1), 4.71 (2H, m, H-2, H-3), 4.41 (1H, septet, NCH), 4.30 (1H, m, H-4), 3.60 - 3.43 (2H, m, 2 x H-5), 1.48 (3H, s, CCH_3), 1.31 (3H, s, CCH_3), 1.16 (3H, d, J = 6.6 Hz, NCHC**H**$_3$), 1.07 (3H, d, J = 6.4 Hz, NCHC**H**$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.11, 144.39, 125.57, 120.77, 120.73 (aromatic carbons), 112.42 (OCO), 104.90 (C-1), 84.12 (d, J = 11.0 Hz, C-2), 81.43 (d, J = 10.1 Hz, C-3), 71.90 (d, J = 5.5 Hz, C-4), 48.43 (d, J = 7.3 Hz, NCH), 39.38 (C-5), 26.58, 26.15 (**Me**$_2$C), 20.86 (d, J = 6.4 Hz, NCH**C**H$_3$), 20.26 (d, J = 2.8 Hz, NCH**C**H$_3$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 61.58; HRMS (FAB, glycerol) m/e calcd for $C_{17}H_{24}N_2O_7PS$ [MH^+] 431.1043, found 431.1042.

Oxazaphosphorinane (73d).

By the procedure described for the preparation of **73a, 73d** was synthesized and its ^{31}P NMR showed one peak at 127.50 ppm, but we can not purify the product by chromatography. Therefore, the reaction mixture was used in the next step.

Phosphorothioamidate (74d).

By the procedure described for the preparation of **74a, 74c** was synthesized and its ^{31}P NMR showed one peak at 66.8 ppm. Unfortunately we also can not purify the product by chromatography.

2.5.4. Experimental for Sections 2.3.4.

2-benzyl-4,5-dicyanoimidazole (83).[24]

To a solution of 2-amino-4,5-dicyanoimidazole (1.33 g, 10 mmol) in 33 mL of water was added 7.5 mL of concentrated HCl and then a solution of $NaNO_2$ (1. mL, 14 mmol) in 25 mL of water over 10 min at rt. After cooling 1 h at 0 ^{0}C, white diazodicyanoimidazole precipitated quantitatively. It was filtered and dried under vacuum. Then the diazodicyanoimidazole (0.7 g, 5 mmol) in 25 mL of benzene was heated under reflux for 2 h, the nitrogen was evolved and 2-phenyl-4,5-dicyanoimidazole **83** (0.8 g, yield 85%) was crystallized from the solution on cooling: m.p.>200 ^{0}C (decomposed); MS (CI-NH_3) m/e 195 ($[M+H]^+$, 100).

2-Bromo-4,5-diethylcarboxylimidazole (84).[25]

A mixture of imidazole-4,5-dicarboxylic acid (1 g, 6.4 mmol), 50 mL of ethanol and 6 mL of H_2SO_4 was refluxed overnight until a homogeneous solution was attained. The mixture was concentrated, the residue was then dissolved in 60 mL of H_2O and the aqueous solution was neutralized with $NaHCO_3$. The mixture was partitioned using four 50 mL portions of ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a white solid. This material was dissolved in ethyl acetate and precipitated by dropwise addition to petroleum ether. Titration and washing with petroleum ether gave a white solid of 4,5-diethylcarboxylimidazole (0.9 g, 64%): m.p., 138 - 145 ^{0}C; MS (EI) m/e 212 (M^+, 28.2)

To a solution of 4,5-diethylcarboxylimidazole (718 mg, 3.38 mmol) in DMF (12 mL) was added in one portion a solution of NBS (723 mg, 1.2 eq) in DMF (12 mL). The mixture was allowed to stir at rt for 1 h and concentrated to dryness. The residue was dissolved in ethyl acetate and the solution was then washed with brine, saturated Na_2SO_3 and brine. The organic layer was dried over $MgSO_4$ and concentrated to dryness to give 2-bromo-

4,5-diethylcarboxylimidazole **84** (735 mg, 75%): m.p. 123 - 130 ^{0}C; MS (EI) 290 (M^+, 48.2).

Benzimidazolium Triflate (86).[26]

To a solution of benzimidazole (1.18 g, 10 mmol) in CH_2Cl_2 was added trifluoromethanesulfonic acid (1.5 g, 1 eq) at rt. After stirring for 15 min., the reaction mixture was filtrated to give light yellow powder of benzimidazolium triflate **86** in quantitative yield: m.p. 204 - 206 ^{0}C (lit. 198 - 200 ^{0}C); MS (FAB, glycerol) m/e 298 ($[M+H]^+$, 20.2).

***p*-nitrophenyltetrazole (87).**[27]

A mixture of 4-nitrobenzonitrile (3.3 g, 22 mmol), sodium azide (1.72 g, 1.2 eq) and ammonium chloride (1.41 g, 1.2 eq) in DMF (20 mL) was stirred and heated at 100 ^{0}C for 10 h. The solvent was removed at reduced pressure and provided yellow solid. Then recrystallization with H_2O gave a yellow solid of *p*-nitrophenyltetrazole **87** (3.8 g, 90%):m.p. 205 - 210 ^{0}C; MS (CI-NH_3) m/e 192 ($[M+H]^+$, 28.7), 163 (100), 135 (72.7).

2-iodo-4,5-dicyanoimidazole (88)

A mixture of 2-bromo-4,5-dicyanoimidazole **46** (197 mg, 1.0 mmol), KI (1.66 g, 10.0 mmol), CuI (0.953 mg, 5.0 mmol) and HMPT (1-methyl-2-pyrrolidinone) (3 mL) was heated at 120 ^{0}C overnight. The reaction was quenched with 2N HCl (20 mL) and then the reaction mixture was taken up with ethyl acetate. The organic layer was washed with aq. Na_2SO_3, 2N HCl and H_2O and dried over $MgSO_4$. After recrystallization with H_2O, a white solid **88** was obtained (166 mg, 68%): m.p. 185 - 190 ^{0}C; MS (FAB, NBA) m/e 245 $(M+H)^+$, (EI) m/e 244; HRMS (EI) m/e calcd for C_5HN_4I $[M]^+$ 243.92478, found 243.92470.

pKa Measurement:

Titrations were performed with a Radiometer PHM63 pH meter equipped with a Radiometer TTT80 titration controller, ABU80 automatic burette and an REC80 chart recorder with an REA160 titration module and a REA270 pH-stat module. Standardized NaOH solutions purchased from Aldrich Chemical Co. were used as titrant. Doubly distilled, degassed water was used to make up the solutions being titrated. The TTA80 titration vessel was purged with a flow of nitrogen. The pH was measured with a Radiometer K-4040 calomel reference electrode and a G-2040C glass electrode.

4,5-dicyanoimidazole **82** (methanol/H_2O = 1/1): pKa = 5.1 (pKa = 5.2)

2-phenyl-4,5-dicyanoimidazole **83** (methanol/H_2O/dioxane = 1/1/1): pKa = 5.3

2-bromo-4,5-diethylcarboxylimidazole **84** (methanol/H_2O = 1/1): pKa = 6.4

2-iodo-4,5-dicyanoimidazole **88** (methanol/H_2O = 1/1): pKa < 3

2.5.5. Experimental for Section 2.3.5.

(S)-1,2,4-Butanetriol (93).

To a stirred solution of 2 M solution of borane-methyl-sulfide complex (24 mL, 48 mmol) and trimethylborate (5 mL) was added dropwise (L)-malic acid **92** (2 g, 14.9 mmol) in THF (10 mL) under argon atmosphere at 0 °C. The cool bath was removed after 5 min and the mixture was stirred overnight at rt. Methanol was added dropwise, the solvent was evaporated and coevaporated with methanol three times afforded 1.94 g of (S)-1,2,4-butanetriol **93**. Without further purification, the crude oil was used directly in the following step.

(S)-1,2-O-cyclohexylidene-1,2,4-butanetriol (94).

To a solution of (S)-1,2,4-butanetriol **93** (1.9 g, 18 mmol) in cyclohexanone (80 mL) was added *p*-toluenesulfonic acid (0.28 g, 1.5 mmol). The mixture was stirred at rt overnight.

Triethylamine (1 mL) was added and the solvent was removed and flash chromatography on silica gel (Ethyl acetate/hexanes = 70/30) to give (S)-1,2-O-cyclohexylidene-1,2,4-butanetriol **94** (2.3 g, 85% yield from **92**): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.20 (m, 1H, H-2), 4.02 (ABX, 1H, H-1), 3.73 (t, J = 5.8 Hz, 2H, H-4), 3.53(ABX, 1H, H-1'), 2.75 (b, 1H, OH), 1.76 (m, 2H, 2 x H-3), 1.57 - 1.32 (m, 10H, cyclohexyl protons); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 109.5 (OCO), 74.6 (C-2), 69.0 (C-1), 60.3 (C-4), 36.4 (C-3), 35.6, 35.0, 25.0, 23.9, 23.7 (cyclohexyl carbons); MS (CI-NH_3) m/e 187 ([M+H]$^+$, 100), 157 (18.4), 143 (89.4), 112 (0.2).

(S)-1,2-O-cyclohexylidene-4-O-mesyl-1,2,4-butanetriol (95).

To a solution of (S)-1,2-O-cyclohexylidene-1,2,4-butanetriol **94** (1.24 g, 6.67 mmol) and triethylamine (9.4 mL, 66.7 mmol) in dichloromethane (10 mL) was added mesyl chloride (2.6 mL, 33.4 mmol) at 0 °C under nitrogen atmosphere. After 10 min, the cooling bath was removed and the mixture was stirred for 1 h until TLC showed the starting material disappeared. Ether was added and the solution was washed with saturated $NaHCO_3$, 1% HCl and brine, then dried over $MgSO_4$. The solvent was evaporated to give an oil of (S)-1,2-O-cyclohexylidene-4-O-mesyl-1,2,4-butanetriol **95** (2.4 g, 93%) which was directly used in the following step: ^{1}H NMR (200MHz, $CDCl_3$) δ 4.35 (m, 2H, H-4), 4.19 (m, 1H, H-2), 4.06 (ABX, 1H, H-1), 3.57 (ABX, 1H, H-1'), 3.01 (s, 3H, OSO_2CH_3), 1.95 (m, 2H, H-3), 1.58 - 1.30 (m, 10H, cyclohexyl protons).

(S)-1,2-O-cyclohexylidene-4-isopropylamino-1,2-butanediol (96).

To a solution of (S)-1,2-O-cyclohexylidene-4-O-mesyl-1,2,4-butanetriol **95** (7.4 g, 28 mmol) in CH_2Cl_2 (5 mL) in a pressure bottle was added isopropylamine (50 mL). The mixture was stirred at 55 °C overnight. The solvent was removed by rotary evaporator and the remaining yellow oil was taken up with CH_2Cl_2 and washed with saturated $NaHCO_3$

and brine, then dried over $MgSO_4$. The solvent was evaporated and the residue was flash chromatographed on silica gel (ethyl acetate/triethylamine = 98/2) to furnish (S)-1,2-O-cyclohexylidene-4-isopropylamino-1,2-butanediol **96** (6.03 g, 95%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.12 (m, 1H, H-2), 4.02 (ABX, 1H, H-1), 3.50 (ABX, 1H, H-1'), 2.80 (m, 1H, NCH), 2.70 (m, 1H, H-4), 1.74 (m, 2H, 2 x H-3), 1.59 - 1.37 (m, 10H, cyclohexylidene protons), 1.07 - 1.05 (dd, 6H, NCH($\mathbf{CH_3}$)$_2$); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 109.50 (OCO), 74.53 (C-2), 69.10 (C-1), 48.78 (C-4), 44.24 (NCH), 33.95 (C-3), 36.53, 35.21, 25.12, 23.85, 23.70 (cyclohexyl carbons), 22.72, 22.58 (NCH($\mathbf{CH_3}$)$_2$); MS (CI-NH_3) m/e 228 ([M+H]$^+$, 100), 184 (28.6), 129 (23.3), 114 (35.9).

(S)-1,2-O-cyclohexylidene-4-(N-isopropyl-N-trifluoroacetyl)amino-1,2-butanediol (97).

To a solution of (S)-1,2-O-cyclohexylidene-4-isopropylamino-1,2-butanediol **96** (11 g, 48.4 mmol) in pyridine (12 mL) was added dropwise trifluoroacetic anhydride (15 mL, 2.2 eq) at 0 °C under nitrogen atmosphere. The reaction was followed with TLC until the starting material disappeared (3 h). The solution was taken up with CH_2Cl_2 and the mixture was washed with $NaHCO_3$ and brine, then dried over $MgSO_4$. The solvent was evaporated to give a yellow oil of (S)-1,2-O-cyclohexylidene-4-(N-isopropyl-N-trifluoroacetyl)amino1,2-butanediol **97** (14.2 g, 91%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.19 (NCH), 4.12 (m, 1H, H-2), 4.03 (ABX, 1H, H-1), 3.55 (ABX, 1H, H-1'), 3.37 (m, 1H, H-4), 1.75 (m, 2H, 2 x H-3), 1.60 - 1.37 (m, 10H, cyclohexylidene protons), 1.07 - 1.05 (d, 6H, NCH($\mathbf{CH_3}$)$_2$); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 156.8 ($\mathbf{C}OCF_3$), 109.7 (OCO), 73.6 (C-2), 68.6 (C-1), 48.9 (C-4), 39.3 (NCH), 32.7 (C-3), 36.6, 35.0, 25.1, 24.0, 23.8 (cyclohexyl carbons), 21.0 (NCH($\mathbf{CH_3}$)$_2$); MS (EI) m/e 323 (M$^+$, 43.2), 280 (100).

(S)-4-(N-isopropyl-N-trifluoroacetyl)amino-1,2-butanediol (91).

To a solution of (S)-1,2-O-cyclohexylidene-4-(N-isopropyl-N-trifluoroacetyl)amino1,2-butanediol **97** (6.7 g, 20.7 mmol) in methanol (50 mL) was added 10% HCl (70 mL). The mixture was stirred at rt for 10 h. The solvent was evaporated and coevaporated with methanol. The crude product was chromatographed on silica gel (CH_2Cl_2/methanol = 9/1) to give a colorless oil of (S)-4-(N-isopropyl-N-trifluoroacetyl)amino-1,2-butanediol **91** (4.5 g, 90%, rotamer ratio is 9 : 1).

The following was assigned to the major rotamer:

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.22 (NCH), 3.69 (m, 1H, H-2), 3.63 (ABX, 1H, H-1), 3.54 (m, 1H, H-4), 3.45 (ABX, 1H, H-1'), 3.39 (m, 1H, H-4'), 2.45 (b, 2H, OH), 1.78 (m, 1H, H-3), 1.64 (m, 1H, H-3') 1.26 - 1.24 (dd, 6H, NCH**Me**$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 156.7 (q, **C**OCF$_3$), 120.00 - 113.05 (q, CO**C**F$_3$), 69.7 (C-2), 66.4 (C-1), 49.1 (C-4), 38.7 (NCH), 32.7 (C-3), 21.2, 21.0 (NCH(**C**H$_3$)$_2$); MS (EI) m/e 243 (M^+, 10), 212(42.1), 126 (100); MS (CI-NH_3) m/e 244 ([M+H]$^+$, 16.7), 212 (21.8), 148 (100), 126 (67).

Cyclic sulfite (98).

To a cooled (0 ^{0}C), magnetically stirred solution of diol **91** (160 mg, 0.65 mmol) and triethylamine (734 μl, 8 eq) and CH_2Cl_2 (3 mL) was added thionyl chloride (72 μl) dropwise over 10 min and the mixture was stirred for 30 min. The reaction mixture was diluted with cold ether (20 mL) and washed with cold water and brine. The organic solution was dried over $MgSO_4$. The solvent was evaporated and dried under vacuum to give a yellow oil of cyclic sulfite **98**(160.43 mg, 84%): MS (CI-NH_3) m/e 307 ([M+H]$^+$ + NH_3, 100), 290 ([M+H]$^+$, 57.2), 226 (67.6).

(S)-4-(N-isopropyl-N-trifluoroacetyl)amino-1-O-tosyl-1,2-butanediol (89).

To a solution of diol **91** (2.09 g, 8.6 mmol) in dry pyridine (60 mL) was added *p*-toluenesulfonic chloride (1.64 g, 1.0 eq) under nitrogen at 0 ^{0}C. The reaction mixture was stirred for 6 h, then the reaction was quenched with H_2O. The excess pyridine was evaporated and coevaporated with toluene three times. The crude product was dissolved in $CHCl_3$, washed with H_2O and dried over $MgSO_4$. After removing the solvent, the mixture was chromatographed on a silica gel column (Hexane:Ethyl acetate = 3:1) to give a colorless oil of (S)-4-(N-isopropyl-N-trifluoroacetyl)amino-1-tosyl-1,2-butanediol **89** (2.7 g, 80%) : ^{1}H NMR (500 MHz, CDCl) δ 7.77 - 7.34 (m, 4H, aromatic protons), 4.19 (septet, 1H, NCH), 3.93 (m, 2H, 2 x H-1), 3.82 (m, 1H, H-2), 3.45 (m, 1H, H-4), 3.34 (m, 1H, H-4'), 3.04 (d, J = 4.5 Hz, 1H, OH), 2.43 (s, 3H, SO_2PhCH_3), 1.82 (m, 1H, H-3), 1.68 (m, 1H, H-3') 1.23 (m, 6H, $NCHMe_2$); MS (FAB, NBA) m/e 398 ($[M+H]^+$, 32.2), 380 (32.2), 302 (100).

(S)-4-(N-isopropyl-N-trifluoroacetyl)amino-1-cyano-2-butanol (99).

To a solution of tosylate compound **89** (592 mg, 1.49 mmol) in dry DMF (10 mL) was added sodium cyanide (73 mg, 1 eq). The reaction mixture was heated at 90 - 100 ^{0}C for 3 h. The solvent was removed, then the residue was dissolved in $CHCl_3$, washed with H_2O and dried over $MgSO_4$. After removing the solvent, the mixture was chromatographed on a silica gel column (Hexane:Ethyl acetate = 2:1) to give a colorless oil of (S)-4-(N-isopropyl-N-trifluoroacetyl)amino-1-cyano-2-butanol **99** (225 mg, 60%) : ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.21 (septet, 1H, NCH), 3.90 (m, 2H, H-2, OH), 3.52 (m, 1H, H-4), 3.36 (m, 1H, H-4'), 2.53 (m, 2H, 2 X H-1), 1.88 (m, 1H, H-3), 1.73 (m, 1H, H-3') 1.24 (m, 6H, $NCHMe_2$); ^{13}C NMR (125.7 MHz, CDCl3) δ 157.3 (q, $COCF_3$), 119.82 - 115.26 (q, $COCF_3$), 117.39 (CN), 65.13 (C-2), 49.16(C-4), 38.34 (NCH), 35.79 (C-1), 25.79 (C-

3), 20.95, 20.72(NCH($\mathbf{CH_3}$)$_2$); MS (CI-NH_3) m/e 253([M+H]$^+$, 85.3), 237 (25.2), 193 (32.4), 157 (100).

(S)-4-isopropylamino-1-cyano-2-butanol (54).

To a solution of **99** (100 mg, 0.4 mmol) in methanol was added 28% NH_4OH (2 mL) at rt. The reaction mixture was stirred at rt for 3 h. Removal of the NH_4OH, the residue was dissolved in $CHCl_3$, washed with H_2O and dried over $MgSO_4$. After removing the solvent, the mixture was chromatographed on a silica gel column (Ethyl acetate/methanol = 10:1) to give a colorless oil of (S)-4-isopropylamino-1-cyano-2-butanol **54** (38 mg, 61%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.87 (b, 2H, NH, OH), 4.14 (septet, 1H, NCH), 3.13 (m, 1H, H-2), 2.90 (m, 2H, 2 x H-4), 2.53 (d, 2H, 2 x H-1), 1.80 (m, 2H, 2 x H-3), 1.17 (d, 6H, NCH$\mathbf{Me_2}$); MS (CI) m/e 157 ([M+H]$^+$, 97.9).

2.7. References

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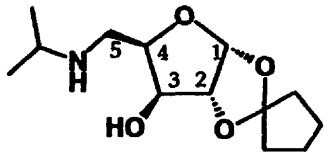
Appendix

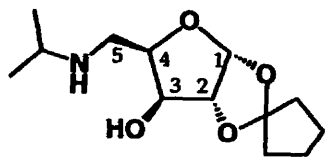
^{1}H NMR and ^{13}C NMR Spectra

^{1}H NMR spectrum of 1,2-O-dicyclopentylidene-5-isopropylamine-α-D-xylofuranose **51**



51

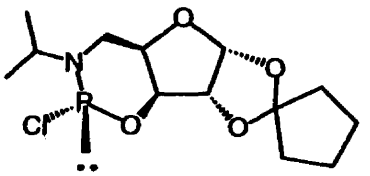
^{13}C NMR spectrum of 1,2-O-dicyclopentylidene-5-isopropylamine-α-D-xylofuranose **51**



51

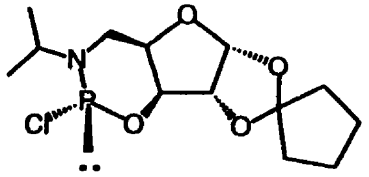
110

^{1}H NMR spectrum of cyclic chlorophosphite **58**



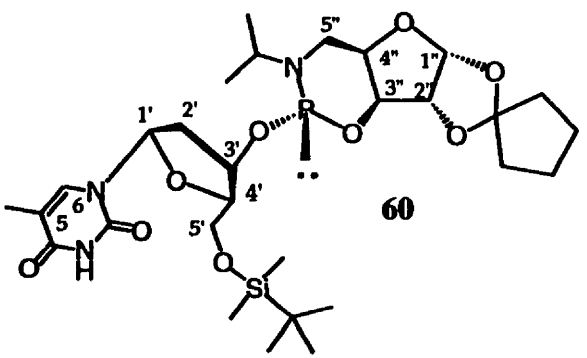
58

^{13}C NMR spectrum of cyclic chlorophosphite **58**



58

^{1}H NMR spectrum of Oxazaphosphorinane **60**



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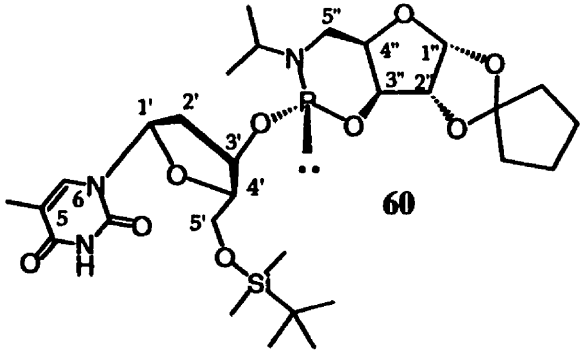
^{13}C NMR spectrum of Oxazaphosphorinane **60**



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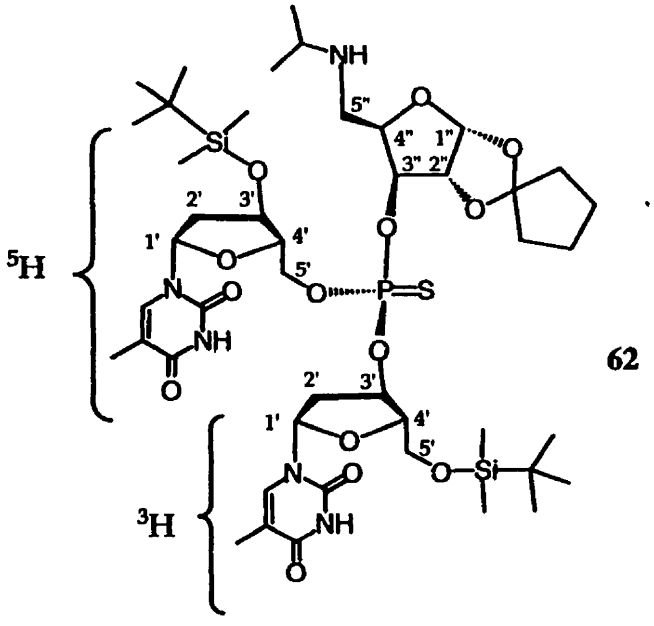
^{1}H NMR spectrum of Protected Phosphorothioate **62**



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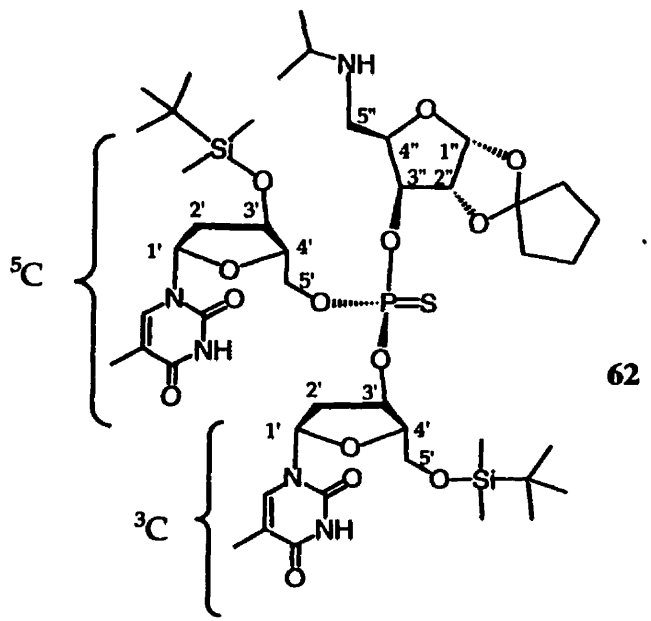
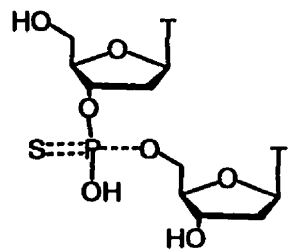
^{13}C NMR spectrum of Protected Phosphorothioate **62**



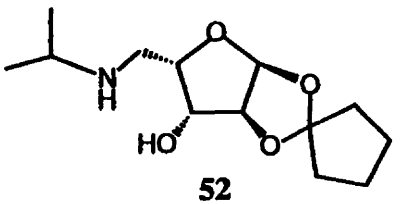
62

^{1}H NMR spectrum of dithymidine phosphorothioate **65**



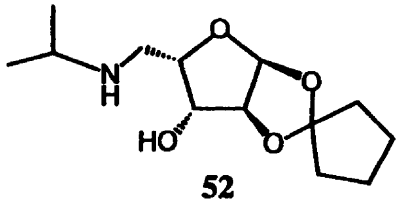
65 (Sp)

^{1}H NMR spectrum of 1,2-O-dicyclopentylidene-5-isopropylamine-α-L-xylofuranose **52**



52

^{13}C NMR spectrum of 1,2-O-dicyclopentylidene-5-isopropylamine-α-L-xylofuranose **52**



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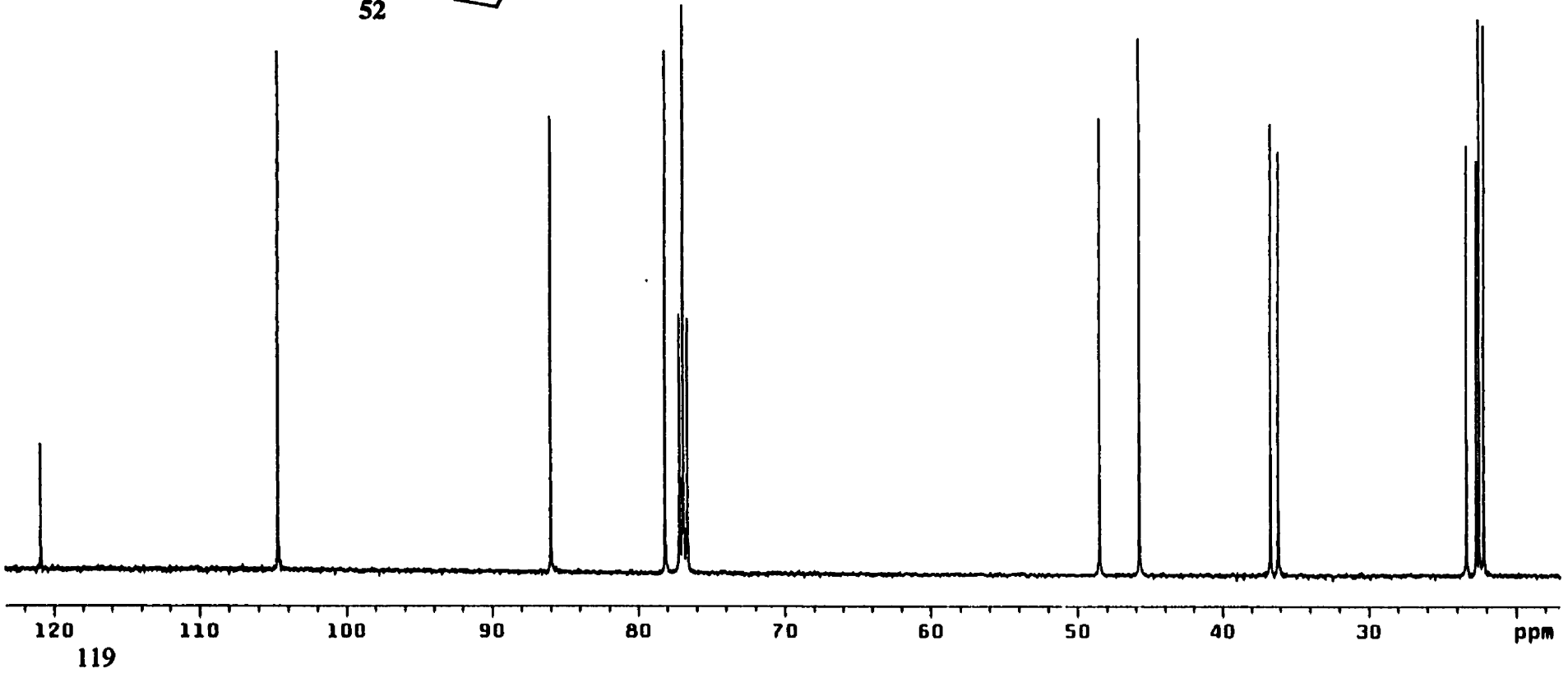
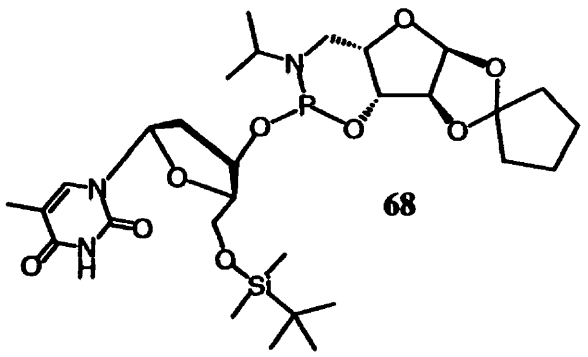


^{1}H NMR spectrum of Oxazaphosphorinane **68**



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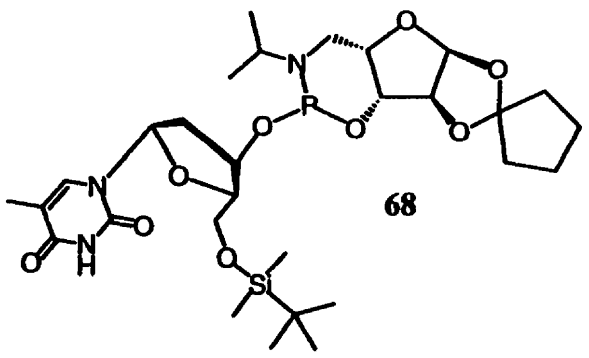
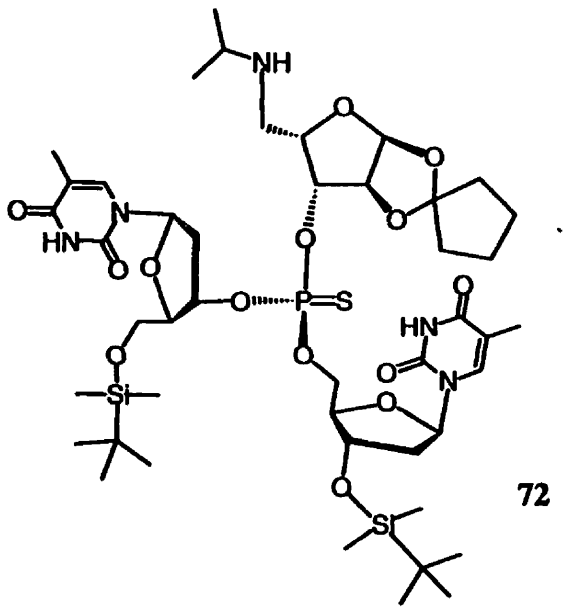
^{13}C NMR spectrum of Oxazaphosphorinane **68**



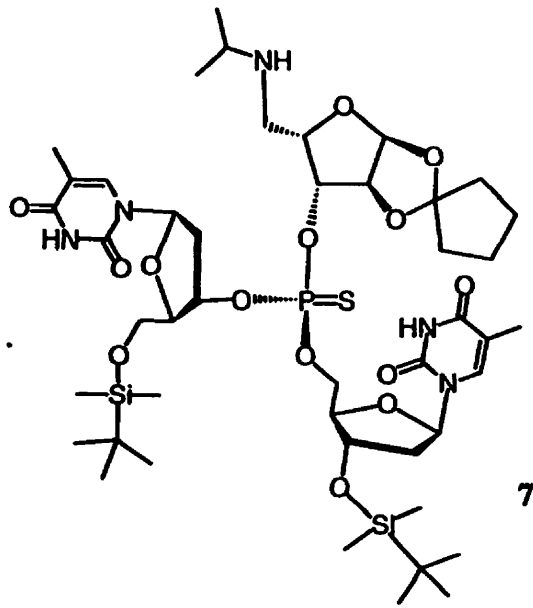
68

^{1}H NMR spectrum of Protected Phosphorothioate **72**



72

^{13}C NMR spectrum of Protected Phosphorothioate **72**



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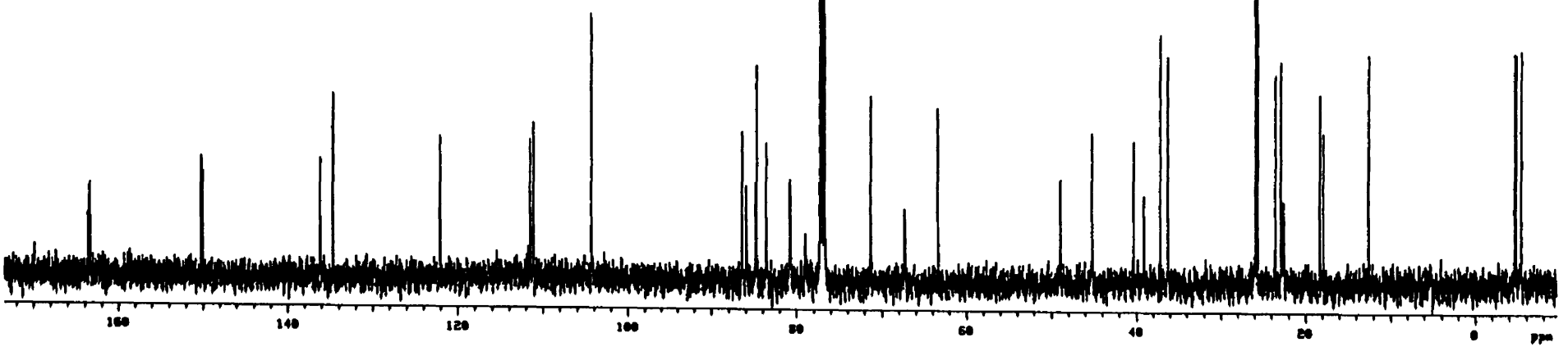
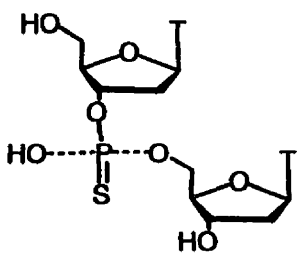
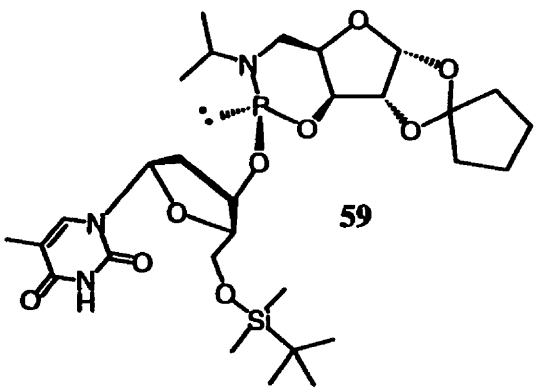


^{1}H NMR spectrum of dithymidine phosphorothioate **66**

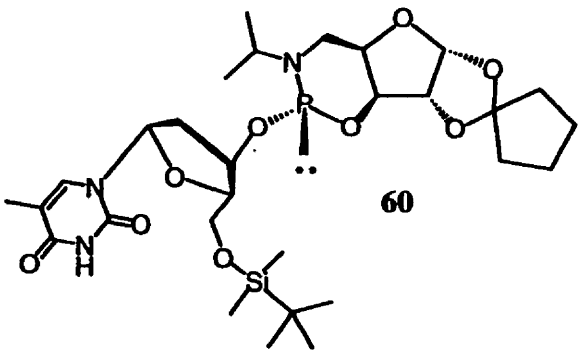


66 (Rp)

^{1}H NMR spectrum of Oxazaphosphorinane: a mixture of 2 : 3 of **59** : **60**



59



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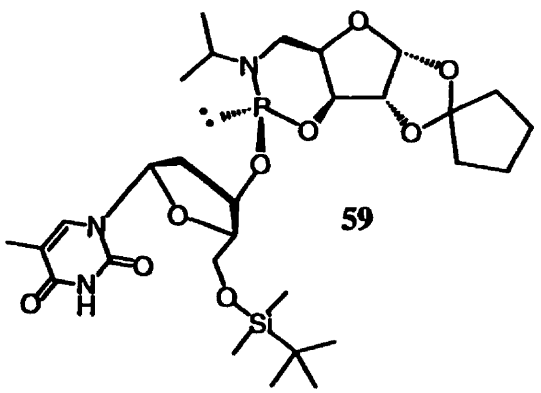
^{13}C NMR spectrum of Oxazaphosphorinane: a mixture of 2 : 3 of **59** : **60**



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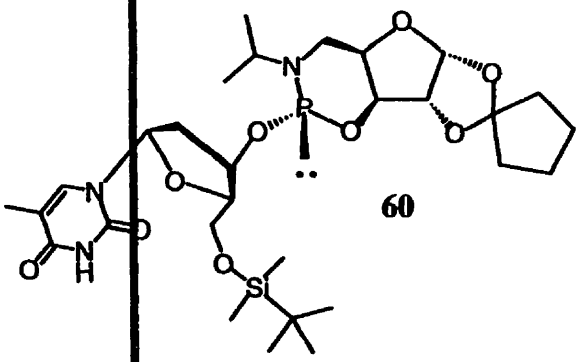

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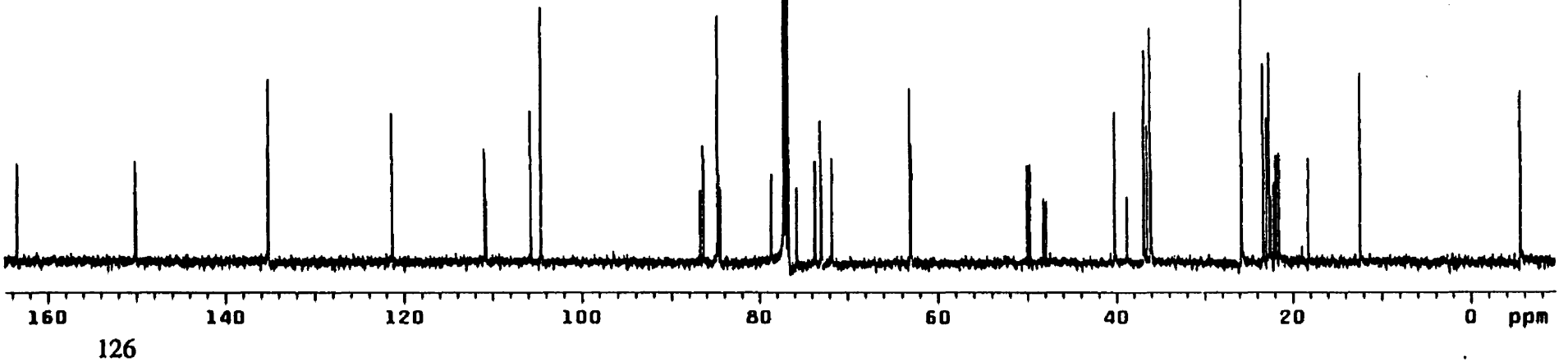
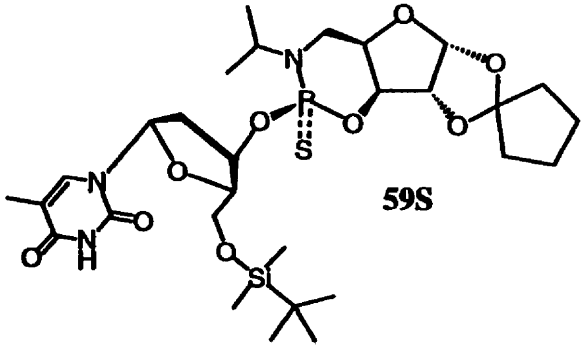
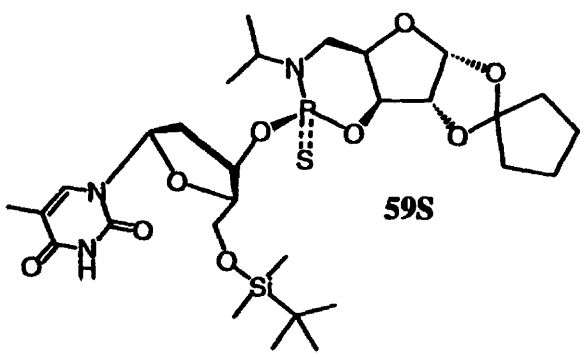


^{1}H NMR spectrum of Thiophosphoramidite **59S**



59S

^{13}C NMR spectrum of Thiophosphoramidite **59S**



59S

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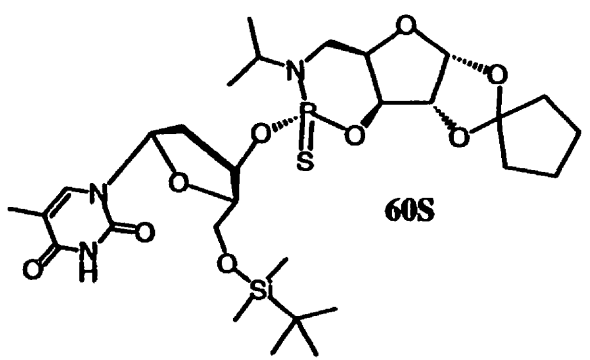
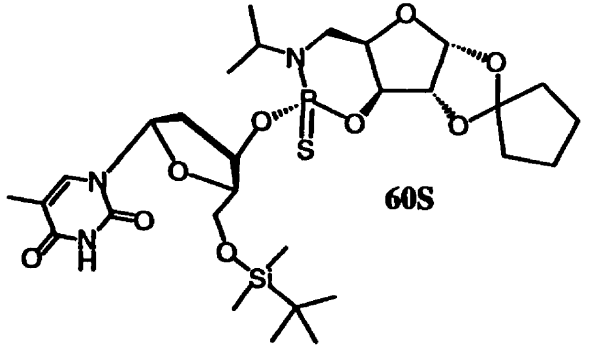


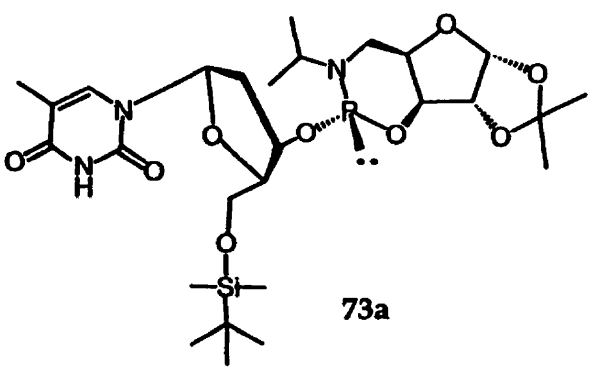
60S

^{13}C NMR spectrum of Thiophosphoramidite **60S**



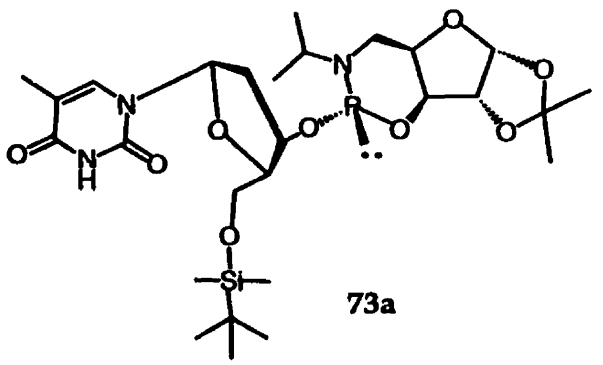
60S

^{1}H NMR spectrum of Thiophosphoramidite **73a**

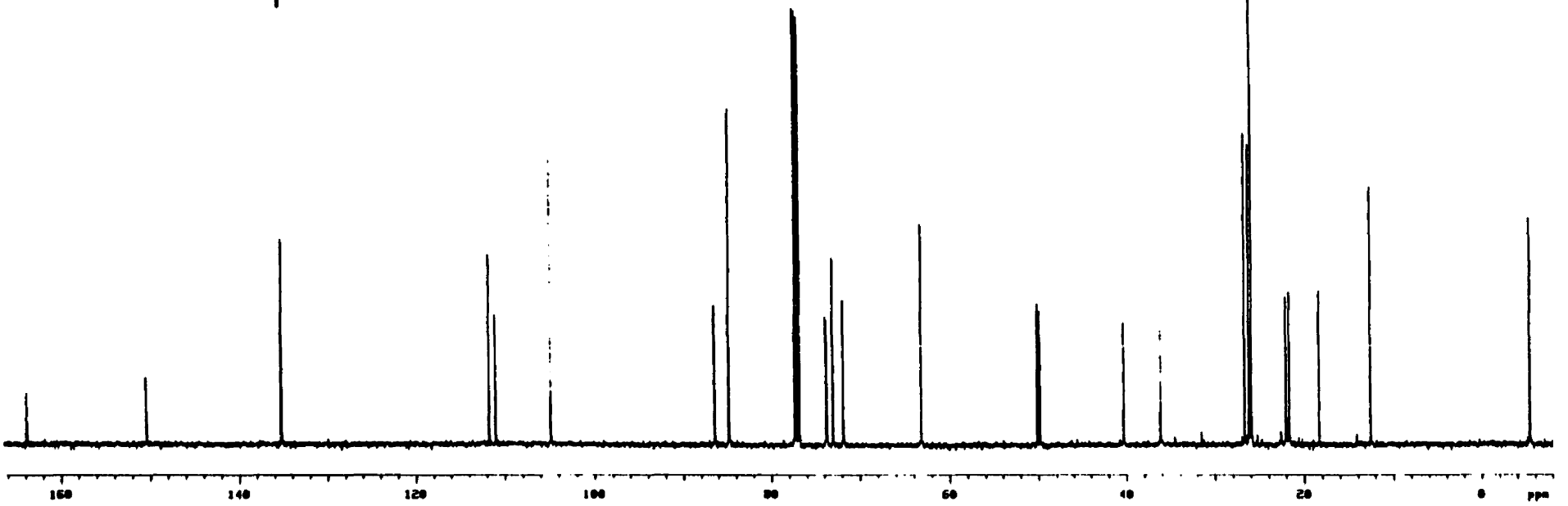


73a

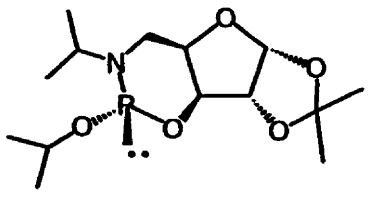
^{13}C NMR spectrum of Thiophosphoramidite **73a**



73a

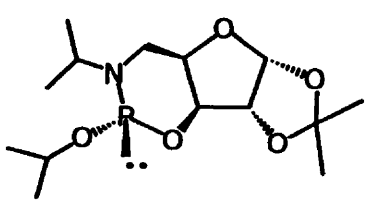


^{1}H NMR spectrum of Thiophosphoramidite **73b**



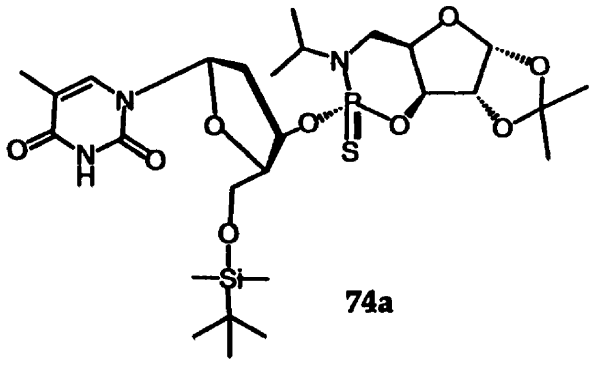
73b

^{13}C NMR spectrum of Thiophosphoramidite **73b**



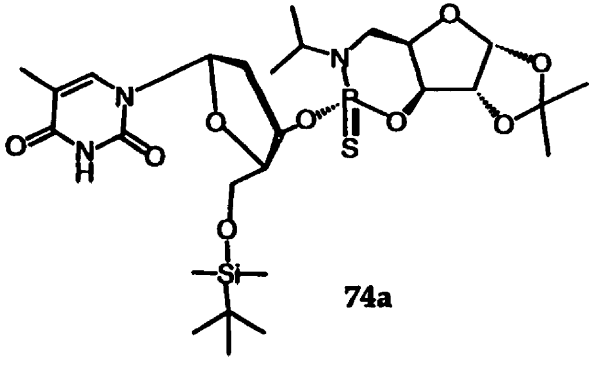
73b

^{1}H NMR spectrum of phosphorothioamidate **74a**



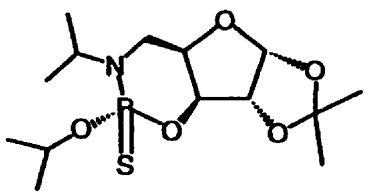
74a

^{13}C NMR spectrum of Phosphorothioamidate**74a**



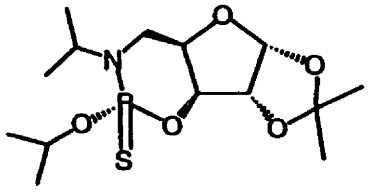
74a

^{1}H NMR spectrum of phosphorothioamidite **74b**



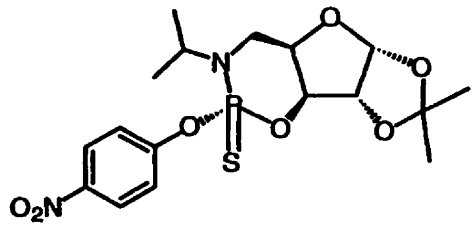
74b

^{13}C NMR spectrum of phosphorothioamidite **74b**



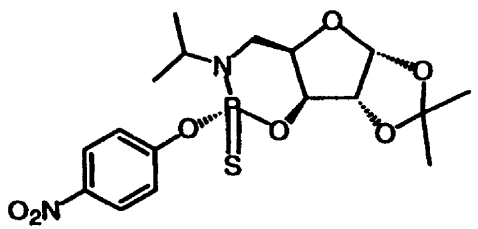
74b

^{1}H NMR spectrum of phosphorothioamidite **74c**



74c

^{13}C NMR spectrum of phosphorothioamidite **74c**



74c

Chapter 3

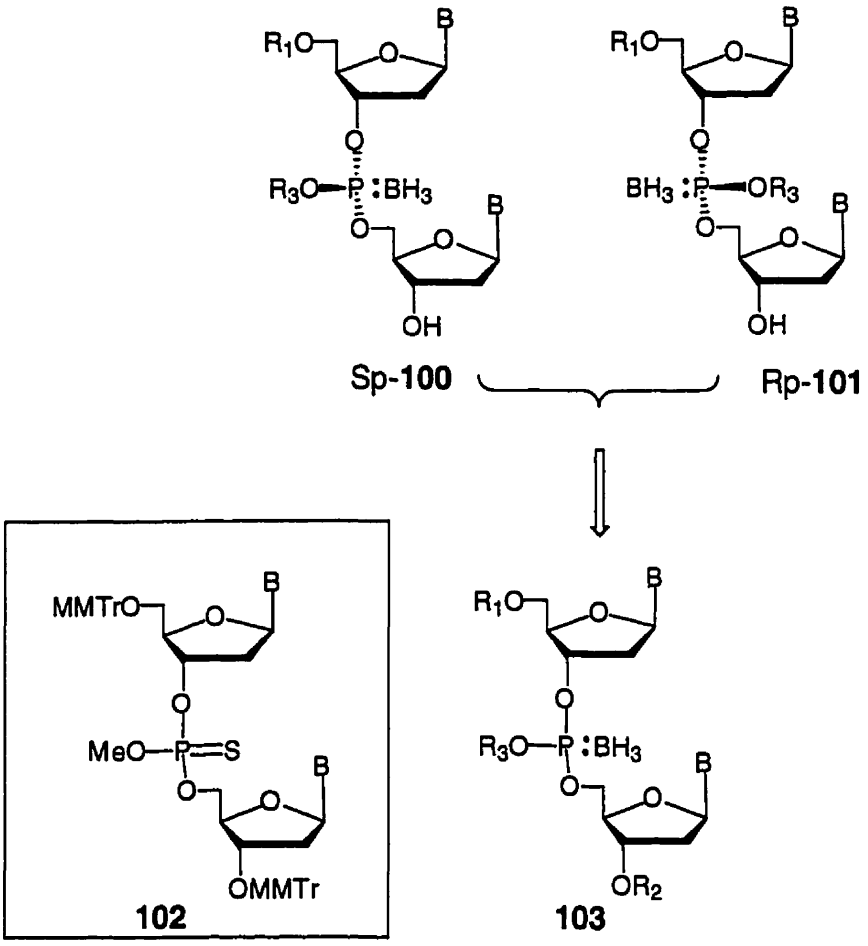
The Synthesis of Sp and Rp Diastereomers of Dithymidine Boranophosphate

3.1. Introduction

Boranophosphates are nuclease resistant[1], and may be very useful antisense agents, in particular since their hybrids with mRNA may be substrates for RNase H. The boranophosphate linkage was first synthesized in dinucleotides by Sood *et al.*[2] Several years later, the separation of the two diastereomers of dithymidine boranomonophosphate using reverse phase HPLC was reported.[3] Recently, a 14mer oligodeoxynucleotide (ODN) containing a single incorporation of one diastereomer of boranophosphate was synthesized enzymatically. This ODN bound to a complementary DNA molecule with slightly poorer binding affinity relative to an unmodified control.[4] Later, Matteucci and coworkers[5] reported the chemical synthesis of a T_{15} ODN fully linked by boranophosphates. They found that the binding affinity of the diastereomeric mixture of the boranophosphate-linked oligothymidine ODN with complementary RNA and DNA was much poorer than that of the native phosphodiester ODN control. Consequently, diastereomeric mixtures of boranophosphates are unlikely to be useful replacements for phosphate diesters in antisense research. The uses of the diastereomerically pure boranophosphates which are not attainable by enzymatic methods, but potentially from stereocontrolled chemical synthesis, remain an open question.

It was recently demonstrated that mixed oligonucleotides showed improved properties as antisense reagents.[6] We will therefore first address the possibility of synthesizing of a mixed backbone oligonucleotides with chiral boranophosphate linkages in this Chapter, since the diastereomerically pure dimers Sp-**100** and Rp-**101** should be easily prepared. It has been reported[7] that two diastereomers of the protected dinucleotide phosphorothioate **102** can be separated easily by chromatography, so it should be possible to separate two diastereomers of the protected dinucleotide boranophosphate **103**, which could be converted to the diastereomerically pure dimers Sp-**100** and Rp-**101** (Scheme 3.1). These dimers then would be incorporated into oligonucleotides to

synthesize mixed backbone oligonucleotides. Then we could attempt to address the effect of stereochemistry of boranophosphate linkage on binding to a complementary strands.



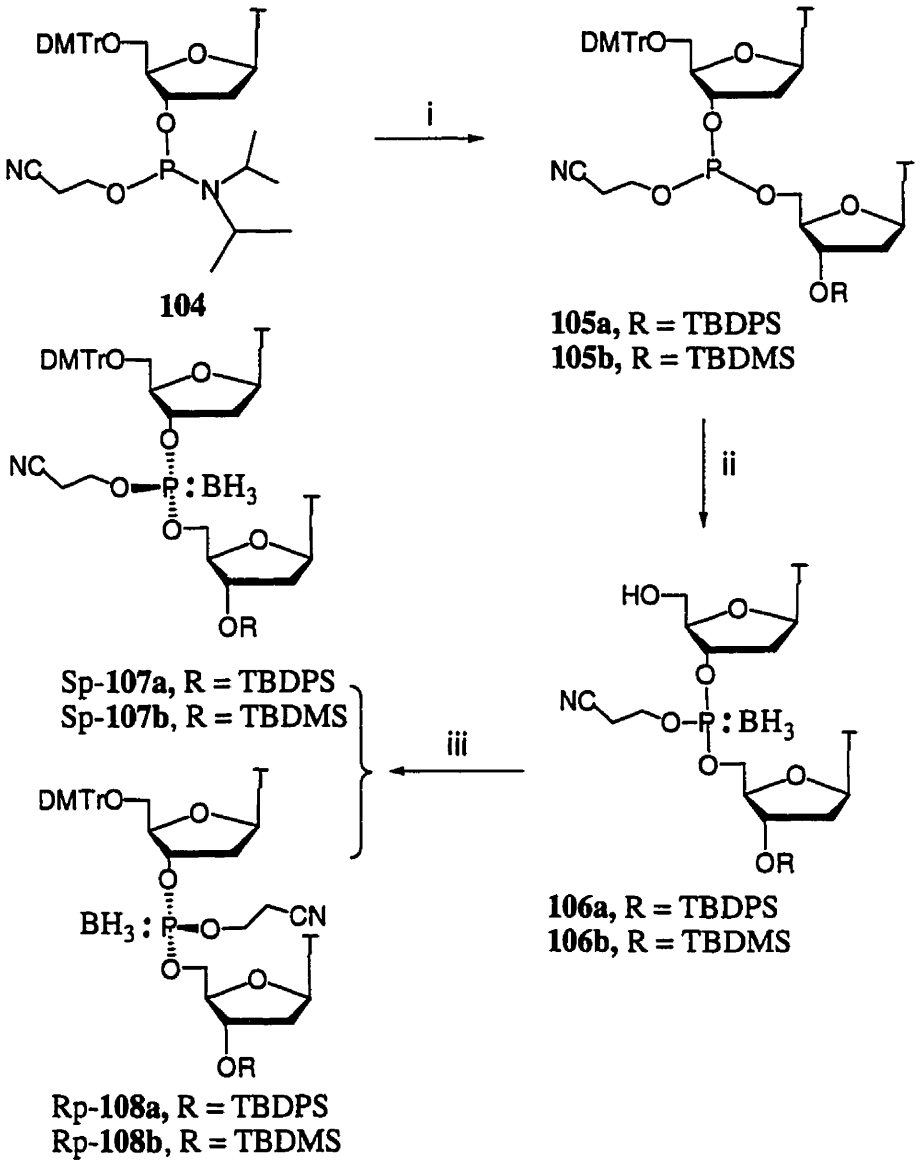
Scheme 3.1: The Possibility of Synthesis of dimers

3.2. Results and Discussion

3.2.1. The Synthesis of dimers Sp-109 and Rp-110

Reaction of commercially available 5'-DMTr-thymidine phosphoramidite **104** with 3'-O-TBDPS thymidine (T_s**OH**) in the presence of tetrazole resulted in the formation of an intermediate phosphite triester **105a**, which was then converted to the dithymidyl boranophosphate cyanoethyl ester **106a** by reaction with excess dimethyl sulfide-borane (Scheme 3.2). Both reactions could be easily followed by ^{31}P NMR. In the first reaction the amidite peaks at 150.88 and 150.82 ppm were replaced by the new phosphite peaks at ~ 140.7 and ~ 140.3 ppm within the time required for recording the spectrum. In the second reaction the phosphite peaks disappeared within 5-10 min; after a large number of accumulations, a broad peak at ~ 117.5 ppm for the boranophosphate phosphorus was observed.

The boronation step with Me_2S-BH_3 also removed the DMTr protecting group from the 5'-hydroxyl position. Separation of **106a** into individual diastereomers was not easy using chromatography. However, after reinstalling the DMTr protecting group at the 5' position in **106a**, the Sp-**107a** and Rp-**108a** diastereomers could be easily separated by chromatography. To get **107a** and **108a** directly from **105a** without detritylation, we tried to use 1 or 2 eq. of Me_2S-BH_3. The reaction did not go to completion and a mixture of **106a**, **107a** and **108a** was obtained. The structures of the diastereomers of Sp-**107a** (yield ~ 45%, R_f = 0.39 - 0.40, fast eluting in ethyl acetate / hexanes = 2 /1) and Rp-**108a** (yield ~ 40%, R_f = 0.25, slow eluting in ethyl acetate / hexanes = 2 /1) were confirmed by ^{1}H, ^{13}C, ^{31}P, ^{11}B NMR and MS.

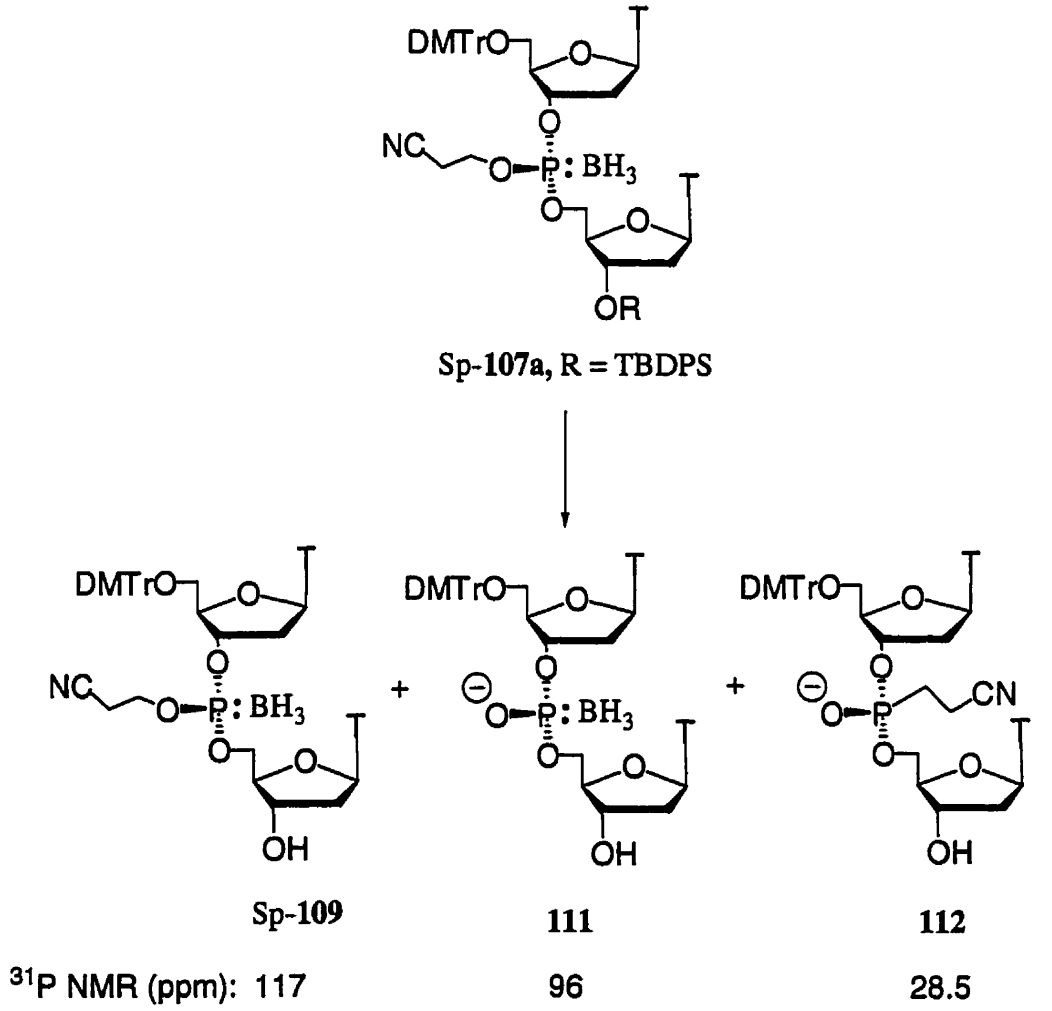


i. T_5'OH, tetrazole, CH_3CN, RT; ii. BH_3-Me_2S in 2.0 M THF; iii. DMTrCl, pyridine,CH_3CN, RT, then chromatography separation.

Scheme 3.2: The Synthesis of Precursors for Dimers Sp-**109** and Rp-**110**

To get dimers Sp-**109** and Rp-**110**, the TBDPS protecting group in 3'-hydroxyl position had to be removed. Different reaction conditions have been investigated (see Table 3.1). In experiment No. 1, TBAF gave desired product Sp-**109** or Rp-**110** which showed ^{31}P NMR at ~ 117 ppm, and a side product which showed ^{31}P NMR peak at ~ 96

ppm. This peak was probably due to decyanoethylated product **111**, since the fluoride anion was quite basic (Scheme 3.3). In experiment No. 4, purification of the final product gave, in addition, the boranophosphates Sp-**109**, **111** and another compound with a ^{31}P NMR at 28.5 ppm, which was characterized as a phosphonate **112**. This compound had lost the BH_3 group, but seemed to have retained its diastereomeric purity since only one single peak was observed in ^{31}P NMR spectrum[8] (Scheme 3.3). The various experiments are summarized in Table 3.1 and the best results for the desilylation were obtained using the protocol outlined in experiment No. 5.



Scheme 3.3: The Removal of Protecting Group in **107a**.

Table 3.1: The Reaction Conditions Used to Remove Protecting Group in **107a** or **108a**

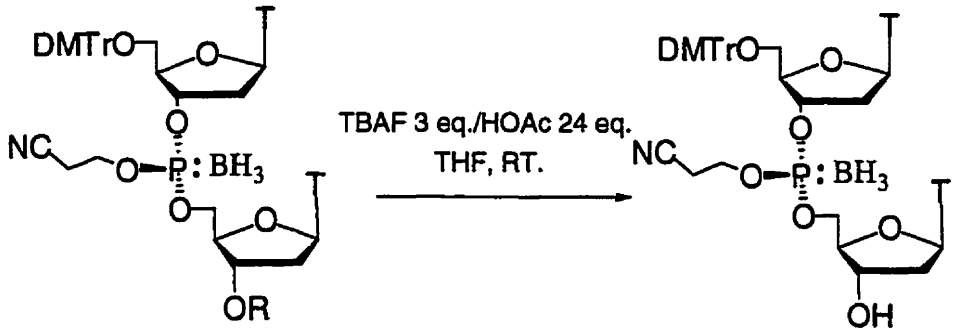
No.	Starting Material	Reaction Condition	Reaction Time	Results (^{31}P NMR ppm)
1	**107a** or **108a**	TBAF 3 eq. in THF	2 min	117 (s), 96 (l),
2	**107a** or **108a**	TBAF 1 eq. in THF	2 min	117 (m), 96 (l),
3	**107a** or **108a**	TBAF 1 eq. /HOAc 1 eq. in THF	20 min	117 (m), 96 (l),
4	**107a** or **108a**	TBAF 3 eq. /HOAc 12 eq. in THF	45 min	117 (l), 96 (s), 28.5 (s)*
5	**107a** or **108a**	TBAF 3 eq. /HOAc 24 eq. in THF	4.5 h	117 (l)
6	**107a** or **108a**	HF / Pyridine in THF		DMTr group was off, reaction was very slow
7	**107a** or **108a**	Et_3N-3HF in THF		Reaction was very slow, TLC showed many spots

l = large peak, m = medium peak, s = small peak,

* only after purification with silica gel column, we could observe the peak at 28.5 ppm which had no BH_3 moiety since no peak was observed in the ^{11}B NMR spectrum, and the loss of the BH_3 moiety was probably due to reaction with fluoride ion.

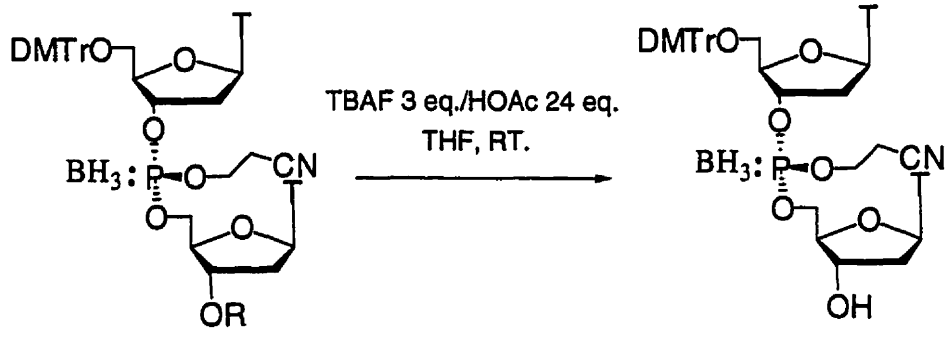
Therefore, each of the diastereomers Sp-**107a** and Rp-**108a** was separately converted with 24 eq. of HOAc and 3 eq. of TBAF in THF at RT to give Sp-**109** (yield: 65 - 70 %) and Rp-**110** (yield: 65 - 70 %) (Scheme 3.4).

Replacement of the TBDPS by a TBDMS group gave similar results. In a parallel way, we synthesized protected dimer **107b** and **108b** (Scheme 3.2). By reacting them with 3 eq. of TBAF and 24 eq of HOAc in THF for 4.5 h, Sp-**107b** and Rp-**108b** were converted to Sp-**109** and Rp-**110** (Scheme 3.4).



Sp-**107a**, R = TBDPS
Sp-**107b**, R = TBDMS

Sp-**109**



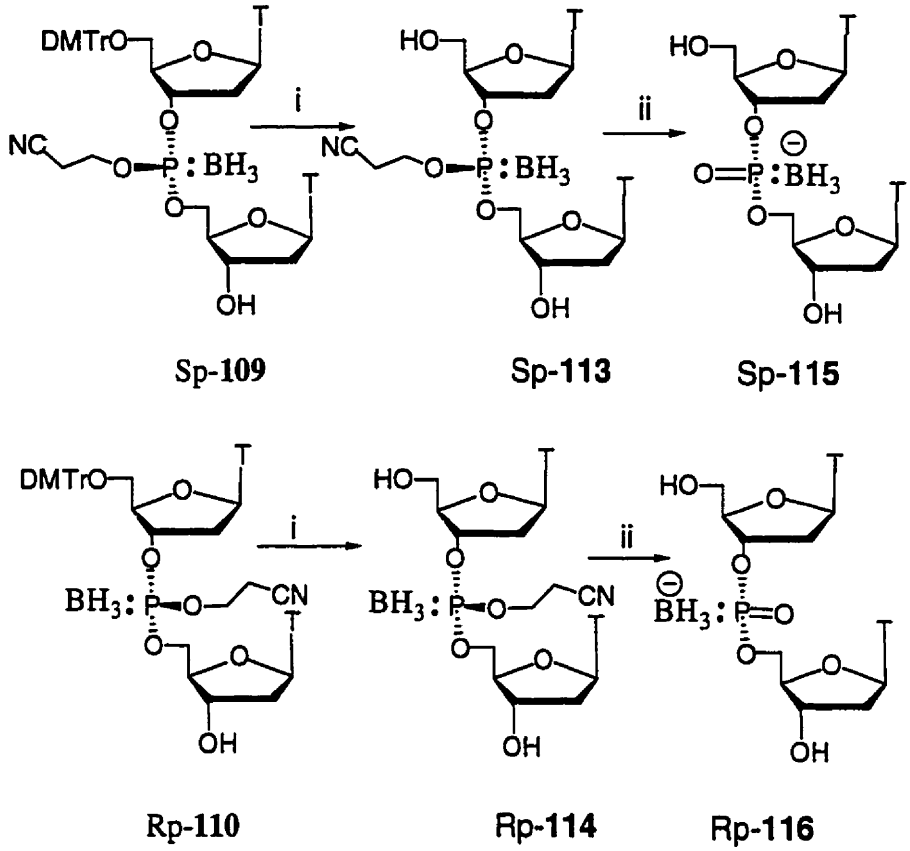
Rp-**108a**, R = TBDPS
Rp-**108b**, R = TBDMS

Rp-**110**

Scheme 3.4: The Synthesis of Dimers Sp-**109** and Rp-**110**

3.2.2. The Synthesis of Sp and Rp Dithymidine Boranophosphates

To confirm the stereochemistry of dimers Sp-**109** and Rp-**110**, we tried to obtain free dithymidine boranophosphates Sp-**115** and Rp-**116**, since the configuration of dimers Sp-**115** and Rp-**116** has been assigned in the literature.[3] Sp-**109** and Rp-**110** gave, after treatment with 70% acetic acid, Sp-**113** (yield: 87%) and Rp-**114** (yield: 90%), respectively. Reaction of Sp-**113** and Rp-**114** with concentrated NH_4OH at RT gave diastereomers Sp-**115** and Rp-**116** in yields of 80% and 75% respectively (Scheme 3.5).



i. 70% HOAc, RT, 2h; ii. 28% NH_4OH, RT, 2h.

Scheme 3.5: The Synthesis of Sp and Rp Dithymidine Boranophosphates

3.2.3. The Structure and Stereochemistry Assignment

Comparison of resistance to enzymatic digestion, ^{31}P NMR chemical shift and retention time in RP HPLC of dinucleotides containing boranophosphate, phosphorothioate and methylphosphonate internucleotide linkage, with respect to the configuration at phosphorus atom of modified internucleotide linkage is shown in Table 3.2.[9]

Table 3.2: Characteristics of Boranophosphate, Phosphorothioate and methylphosphonate Internucleotide Linkage

Internucleotide linkage	Resistance to Enzymatic Digestion[a]	^{31}P NMR (δ)	RP HPLC[e]	Absolute Configuration at Phosphorus
$>P(O)BH_3$	lower[b]	higher field[d]	Fast	Sp
$>PBH_3(O)$	higher[b]	lower field[d]	Slow	Rp
>PS(O)	higher[b]	higher field[d]	Fast	Sp
>P(O)S	lower[b]	lower field[d]	Slow	Rp
$>P(O)CH_3$	stable[b,c]	higher field[d]	Slow	Sp
$>PCH_3(O)$	stable[b,c]	lower field[d]	Fast	Rp

[a] Deprotected, [b]SVPDE: Snake Venom Phosphodiesterase, [c]BSPDE: Calf Spleen Phosphodiesterase, [d]3'- and 5'-protected, [e]isomer Slow eluting: characterized by higher R_f on RP HPLC column, isomer Fast eluting: characterized by lower R_f on RP HPLC column.

It should be pointed out that sulfur is the largest atom around the phosphorus center in a nucleotide phosphorothioate while boron or methyl is the smallest atom around the phosphorus center in a nucleotide boranophosphate or methylphosphonate because of the rule of Cahn-Ingold-Prelog. A Rp configuration in a phosphorothioate corresponds to an Sp configuration in a boranophosphate and methylphosphonate.

The absolute configuration at phosphorus atom in the diastereomers of uridine (3',5')uridine boranophosphate **40** (see Scheme 1.12) was tentatively assigned by the enzymatic digestion with snake venom phosphodiesterase (SVPDE). The resistance of dinucleoside phosphorothioate towards hydrolysis of SVPDE is indicative of Sp configuration, while susceptibility to hydrolysis indicates the Rp configuration.[10] Thus, based on enzymatic criteria, and assuming an isoelectronic structure of boranophosphates with phosphorothioates,[1] the Sp configuration was assigned to the isomer of uridine(3',5')uridine boranophosphate more susceptible to SVPDE digestion. Consequently, the opposite configuration was ascribed to the dimer more resistant to

SVPDE cleavage. This correlation is consistent with the tentative absolute configuration assignment done by means of 1D NOE difference experiments with individual diastereomers of thymidine (3',5')thymidine boranophosphate Sp-d(T_p^BT)-1 and Rp-d(T_p^BT)-2 (see Figure 1.15), and independent SVPDE digestion experiments.[3]

Furthermore, the ^1H NMR spectra of individual diastereomers of thymidine(3',5')thymidine boranophosphate Sp-d(T_p^BT)-1 and Rp-d(T_p^BT)-2 have been reported[3] as shown in Figure 3.1. The precise chemical shift data of each resonance are described in Table 3.3.

The structures of Sp-**115** and Rp-**116** were assigned and compared with the literature data[3]. The configuration at phosphorus in both diastereomers Sp-**115** and Rp-**116** was assigned by ^1H NMR comparison with literature data (Figure 3.1 and Table 3.3).

Since the β-elimination leading from boranophosphate cyanoethyl ester **113/114** to the free boranophosphates **115/116** does not involve any change in stereochemistry at the phosphorus atom, all intermediates have the stereochemistry as depicted as Scheme 3.2, Scheme 3.4 and Scheme 3.5.

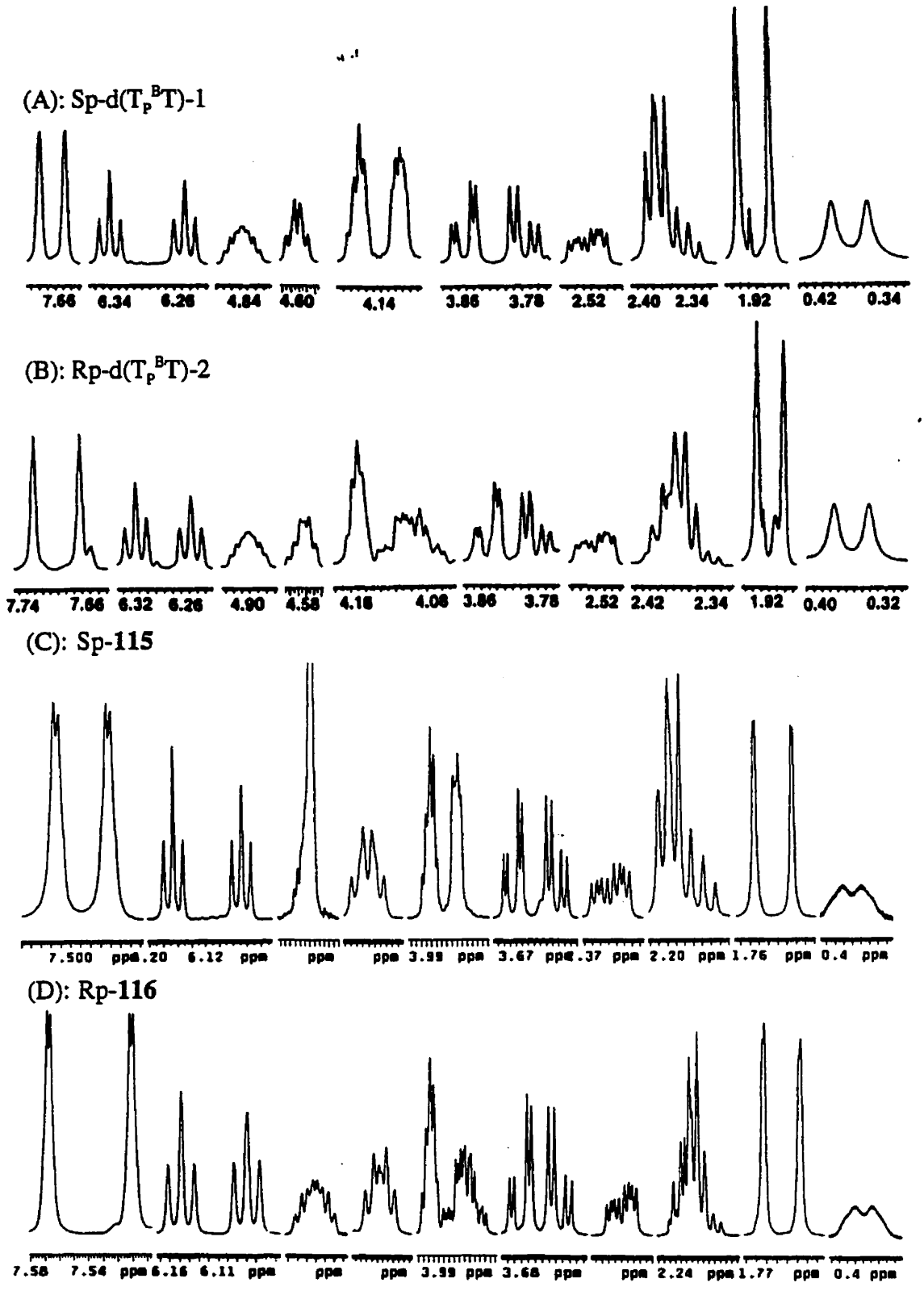


Figure 3.1: (A) and (B): ^{11}B-decoupled ^{1}H NMR spectra of Sp-d(T$_p$^{B}T)-1 and Rp-d(T$_p$^{B}T)-2 at 30 ^{0}C. The spectra were recorded in 100 mM NaCl, 0.1 mM EDTA, 10 mM potassium phosphate, pH 7.4 at 2 mM dimer, the chemical shifts are expressed in ppm relative to the 3-trimethylsilylpropionate-2,3,3,3,-d$_4$ sodium salt (TPS) as internal reference.[3] (C) and (D): ^{1}H NMR spectra of Sp-**115** and Rp-**116** at RT. The spectra were recorded in D_2O, the chemical shifts are expressed in ppm relative to the internal tetramethylsilane (TMS).

Table 3.3: Chemical Shifts for Dithymidine Boranophosphates

	Chemical Shifts (ppm)			
	Sp-**115**[*]	Sp-d(T_P^BT)-1[**]	Rp-**116**[*]	Rp-d(T_P^BT)-2[**]
T_3,O-				
H-1'	6.07	6.246	6.08	6.247
H-2'	2.33	2.514	2.36	2.522
H-2''	2.18	2.351	2.19	2.381
H-3'	4.66	4.836	4.73	4.892
H-4'	3.99	4.160	4.02	4.172
H-5'	3.67	3.848	3.68	3.841
H-5''	3,61	3.777	3.63	3.785
H-6	7.48	7.649	7.51	7.663
TCH_3	1.71	1.892	1.73	1.892
T_5,O-				
H-1'	6.17	6.344	6.15	6.314
H-2'	2.22	2.376	2.24	2.389
H-2''	2.22	2.376	2.21	2.357
H-3'	4.42	4.592	4.41	4.571
H-4'	3.97	4.152	4.00	4.166
H-5'	3.94	4.110	3.96	4.120
H-5''	3.92	4.096	3.92	4.080
H-6	7.51	7.680	7.57	7.722
TCH_3	1.75	1.931	1.76	1.927

*^{1}H NMR spectra of Sp-**115** and Rp-**116** were recorded in D_2O, the chemical shifts are expressed in ppm relative to the internal tetramethylsilane (TMS). ** ^{11}B-decoupled ^{1}H NMR spectra of Sp-d(T_P^BT)-1 and Rp-d(T_P^BT)-2 were recorded in 100 mM NaCl, 0.1 mM EDTA, 10 mM potassium phosphate, pH 7.4 at 2 mM dimer, the chemical shifts are expressed in ppm relative to the 3-trimethylsilylpropionate-2,3,3,3,-d_4 sodium salt (TPS) as internal reference.[3]

3.3. Conclusion

The dithymidine boranophosphate Sp-**109** and Rp-**110** have been synthesized. The configuration at P atom was assigned by converting dimers Sp-**109** and Rp-**110** to the free dithymidine boranophosphates Sp-**115** and Rp-**116**. The other dimers with different bases (A, C, G) should be obtainable using similar method. These dimers will be incorporated into solid-phase synthesis of mixed backbone oligonucleotides. This research is still in progress.

3.4. Experimental

General Materials and Methods

^{1}H NMR spectra were recorded on Varian UNITY 500 Spectrometer at 500 MHz. ^{13}C NMR spectra were recorded at 125.7 MHz on Varian UNITY 500 spectrometer and ^{1}H and ^{13}C NMR chemical shifts are expressed in ppm relative to the internal tetramethylsilane (TMS). ^{31}P NMR spectra were taken on a Varian XL 300, UNITY 500 spectrometers at 121 and 202 MHz with proton-decoupling ({1H}). Positive ^{31}P NMR chemical shifts are expressed in ppm downfield from external 85% H_3PO_4. ^{11}B NMR spectra were recorded on Varian XL 300, UNITY 500 spectrometers at 96.2, 160.4 MHz. ^{11}B NMR chemical shifts were referenced externally to a solution of diethyletherboron trifluoride Et_2O-BF_3. Spin multiplicites are given with the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad peak.

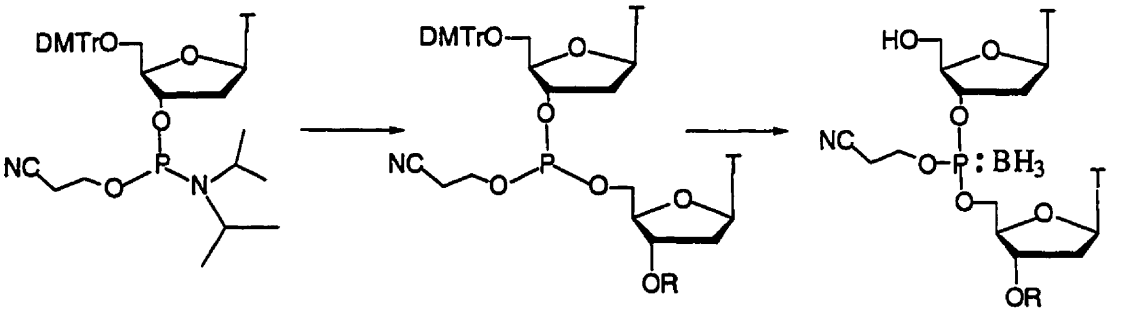
Low resolution mass spectra were recorded on a KRATOS MS 25RFA mass spectrometer in the direct-inlet mode. High resolution mass spectra were performed on a ZAB 2F HS mass spectrometer. Negative electrospray mass spectrometry was performed on the Quattro II triple quadruple mass spectrometer in negative mode.

THF was distilled from sodium benzophenone ketyl, triethylamine and acetonitrile from CaH_2. Pyridine was refluxed for 4 h with fine BaO and distilled over granular BaO under N_2. Anhydrous DMF was purchased from Aldrich in sure-seal bottles and used with no further drying. 3'-O-TBDPS-thymidine, tetrazole were given by Isis Pharmaceuticals. 5'-O-DMTr-thymidine phosphoramidite **104** was a gift from Dalton Chemical Laboratories Inc., Toronto. All other chemicals were purchased from Aldrich Chemical Company Inc. and were used without further purification.

Thin-layer chromatography (TLC) was performed using Kieselgel 60 F_{254} aluminum backed plates (0.2 mm thickness). Column chromatography was performed on 230 - 400 mesh silica gel (Merck).

All air sensitive experiments were performed under dry argon with freshly distilled anhydrous solvents and glassware previously dried overnight on an oven.

Protected Dithymidine Boranophosphate Triesters (106a and 106b).



104

105a, R = TBDPS
105b, R = TBDMS

106a, R = TBDPS
106b, R = TBDMS

Tetrazole (235 mg, 5 eq.) was dissolved in freshly dried CH_3CN (5 ml) under Ar at RT. To this solution, 5'-O-DMTr-thymidine phosphoramidite **104** (500 mg, 0.67 mmol) dissolved in CH_3CN (5 ml) was added by a syringe. The amidite vial was rinsed with another 3 - 4 ml of CH_3CN. 3'-O-TBDPS-thymidine (322 mg, 1 eq) or 3'-O-TBDMS-thymidine (239 mg, 1 eq) was added to the reaction mixture, and the mixture was stirred at RT to give phosphite triester **105a** or **105b**. After 0.5 h, dimethylsulfide-borane in THF

(1.0 ml, 2.0 M) was added, and the mixture was stirred for 10 min. A small portion of the reaction mixture was taken in $CDCl_3$ for ^{31}P NMR determination, which showed complete disappearance of the phosphite resonances and after a large number of accumulations, the appearance of a broad peak at 117.5 ppm for boranophosphate **106**. After 5 h, solvent was removed from the reaction mixture at RT under reduced pressure. The residue was purified by flash chromatography on silica gel (eluent: EtOAc : Hexane = 2 / 1 - 1/0). Yield, 80 - 85%.

106a: $R_f = 0.18$ (Eluent: ethyl acetate)

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.32 ppm;

^{11}B NMR (160.4 MHz, $CDCl_3$) δ - 44.99 ppm;

LRMS (FAB-NBA) m/e 836 ($[M+H]^+$, 12.3);

^1H NMR (500 MHz, $CDCl_3$) δ 9.53, 9.33, 9.32, 9.30 (4s, 2H, NH of two isomers), 7.64 - 7.39 (m, 10H, aromatic protons), 7.42 (s, 1H, ^5H-6), 7.02, 6.96 (2s 1H, ^3H-6 of two isomers), 6.33 , 6.29 (2t, 1H, J = 6.8 Hz, ^5H-1' of two isomers), 6.12 (t, J=6.6 Hz, 1H, ^3H-1'), 5.04 (m, 0.5H, ^5H-3' of one isomer), 4.95 (m, 0.5H, ^5H-3' of one isomer), 4.33 (m, 1H, ^3H-3'), 4.13 - 3.98 (m, 4H, ^5H-4', ^3H-4', CH_2O), 3.96 - 3.90 (m, 1H, ^5H-5'), 3.83 - 3.73 (AB, 2H, ^3H-5', ^3H-5''), 3.71 - 3.67 (m, 1H, ^5H-5''), 3.09, 2.96 (2t, 1H, OH), 2.68 - 2.60 (m, 2H, CH_2CN),), 2.41 - 2.35 (m, 1H, ^3H-2'), 2.33 - 2.29 (m, 2H, ^3H-2'', ^5H-2'), 2.04 - 1.95 (m, 1H, ^5H-2''), 1.88 (s, 3H, $^5CH_3C$-5), 1.86 (s, 3H, $^3CH_3C$-5), 1.06 (s, 9H, $SiMe_3$), 0.38 (b, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^5H-6, $^5CH_3C$-5), (^3H-6, $^3CH_3C$-5), (^5H-1', ^5H-2'), (^5H-1', ^5H-2''), (^3H-1', ^3H-2'), (^3H-1', ^3H-2''), (^5H-3', ^5H-2'), (^5H-3', ^5H-2''), (^5H-3', ^5H-4'), (^3H-3', ^3H-2'), (^3H-3', ^3H-2''), (^3H-3', ^3H-4'), (CH_2O, CH_2CN), (^3H-4', ^3H-5'), (^3H-4',

^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{3}H-5',OH), (^{3}H-5'', OH), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.09, 164.03, 163.99, 163.97 (^{5}C-4 and ^{3}C-4 of two isomers), 150.58, 150.55, 150.42 (^{5}C-2 and ^{3}C-2 of two isomers), 136.63, 136.55 (^{5}C-6 of two isomers), 135.65, 135.53 (^{3}C-6 of two isomers), 135.69, 135.64, 132.82, 132.79, 132.63, 132.60, 130.34, 130.25, 128.09, 128.02 (aromatic carbons), 116.51, 116.31 ($CH_2\underline{C}N$ of two isomers), 111.77, 111.52, 111.32, 111.26 (^{5}C-5 and ^{3}C-5 of two isomers), 86.16, 86.11 (^{3}C-1' of two isomers), 85.26, 84.85 (^{5}C-1' of two isomers), 85.73, 85.62 (2d, ^{5}C-4' of two isomers), 84.61, 84.40 (2d, ^{3}C-4' of two isomers), 78.52, 78.19 (^{5}C-3' of two isomers), 72.48, 72.26 (^{3}C-3' of two isomers), 65.70, 65.30 (2d, ^{5}C-5' of two isomers), 61.90, 61.85 (^{3}C-5' of two isomers), 61.55, 61.42 (CH_2O of two isomers), 39.73, 39.57 (^{5}C-2' of two isomers), 38.55 (^{5}C-2'), 26.79 (SiC$\underline{Me}_3$), 19.68, 19.63 (2d, $\underline{C}H_2CN$ of two isomers), 18.95 (Si$\underline{C}Me_3$), 12.49, 12.43 ($^{3}\underline{C}H_3$C-5, $^{5}\underline{C}H_3$C-5);

HMQC (500 MHz, $CDCl_3$) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), ($C\underline{H_2}O$, $\underline{C}H_2O$), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), ($C\underline{H_2}CN$, $\underline{C}H_2CN$), ($^{3}C\underline{H_3}$C-5, $^{3}\underline{C}H_3$C-5), ($^{5}C\underline{H_3}$C-5, $^{5}\underline{C}H_3$C-5).

106b: R_f = 0.20 (Eluent: ethyl acetate);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.47 ppm;

^{11}B NMR (160.4 MHz, $CDCl_3$) δ - 44.23 ppm;

LRMS (FAB-NBA) m/e 712 ($[M+H]^+$, 38.0);

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85, 9.69 (2s, 1H, NH of two isomers), 9.65, 9.61 (2s, 1H, NH of two isomers), 7.48, 7.47 (2s, 1H, ^{5}H-6 of two isomers), 7.17, 7.15 (2s 1H, ^{3}H-6 of two isomers), 6.21 - 6.14 (m, 2H, ^{5}H-1', ^{3}H-1'), 5.14 (m, 0.4H, ^{5}H-3' of one isomer), 5.07 (m, 0.6H, ^{5}H-3' of one isomer), 4.40 (m, 1H, ^{3}H-3'), 4.29 - 4.18 (m, 4H, CH_2O, ^{3}H-5', ^{3}H-5''), 4.17 (m, 1H, ^{5}H-4'), 3.96 (m, 1H, ^{3}H-4'), 3.84 - 3.77 (m, 2H, ^{5}H-5', OH), 3.73 - 3.29 (m, 1H, ^{5}H-5''), 2.79 - 2.76 (m, 2H, CH_2CN),), 2.46 - 2.37 (m, 2H, ^{5}H-2', ^{5}H-2''), 2.26 (m, 1H, ^{3}H-2', ^{3}H-2''), 1.92, 1.90 (2s, 3H, $^5CH_3$C-5 of two isomers), 1.85, 1.83 (2s, 3H, $^3CH_3$C-5 of two isomers), 0.87 (s, 9H, $SiCMe_3$), 0.48 (b, 3H, BH_3), 0.074, 0.069 (d, 6H, $SiMe_2$);

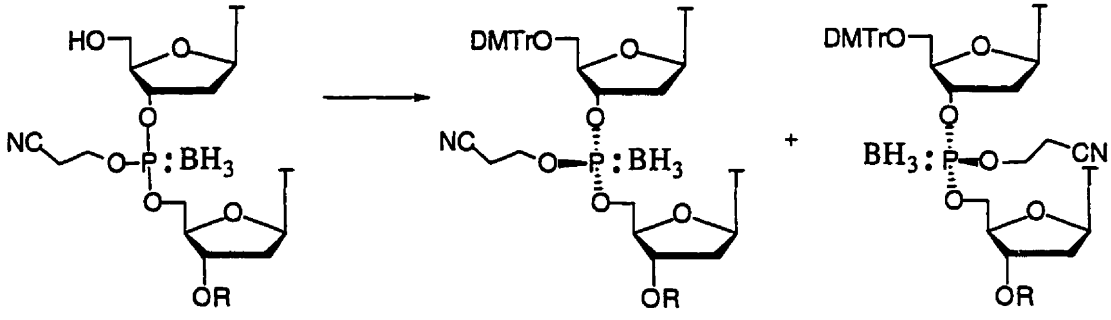
COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (CH_2O, CH_2CN), (^{5}H-4', ^{5}H -5'), (^{5}H-5', ^{5}H-5''), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H -5''), (^{5}H-5', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.25, 164.16 (^{5}C-4 of two isomers), 164.09, 164.03 (^{3}C-4 of two isomers), 150.64, 150.62 (^{5}C-2 of two isomers), 150.53, 150.40 (^{3}C-2 of two isomers), 136.64, 136.62 (^{5}C-6 of two isomers), 136.31, 136.12 (^{3}C-6 of two isomers), 116.68, 116.47 (CH_2CN of two isomers), 111.62, 111.42 (^{5}C-5 of two isomers), 111.32, 111.27 (^{3}C-5 of two isomers), 86.23, 86.14, 85.55 (^{5}C-1', ^{3}C-1' of two isomers), 85.86, 85.79 (2d, ^{5}C-4' of two isomers), 84.58, 84.42 (2d, ^{3}C-4' of two isomers), 78.60, 78.34 (^{5}C-3' of two isomers), 71.10, 71.01 (^{3}C-3' of two isomers),

65.74, 65.48 (2d, ^{3}C-5' of two isomers), 61.94, 61.74 (^{5}C-5' of two isomers), 61.64 (CH_2O), 55.26 (OCH_3), 39.90, 39.82 (^{3}C-2' of two isomers), 38.65 (^{5}C-2'), 25.64 (SiC<u>Me</u>$_3$), 19.81, 19.76 (2d, <u>C</u>H_2CN of two isomers), 17.87 (Si<u>C</u>Me$_3$), 12.56, 12.46 (^{3}<u>C</u>H_3C-5, ^{5}<u>C</u>H_3C-5), -4.68, -4.88 (Si<u>Me</u>$_2$);

HMQC (500 MHz, $CDCl_3$): (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (C<u>H</u>$_2$O, <u>C</u>H_2O), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (C<u>H</u>$_2$CN, <u>C</u>H_2CN), (^{3}C<u>H</u>$_3$C-5, ^{3}<u>C</u>H_3C-5), (^{5}C<u>H</u>$_3$C-5, ^{5}<u>C</u>H_3C-5), (SiC<u>Me</u>$_3$, SiC<u>Me</u>$_3$).

Dithymidine Boranophosphate Triesters (107a, 107b, 108a, 108b).



106a, R = TBDPS
106b, R = TBDMS

107a, R = TBDPS
107b, R = TBDMS

108a, R = TBDPS
108b, R = TBDMS

To a solution of **106a** or **107b** (1.0 mmol) and DMTrCl (2 eq) in CH_3CN, was added pyridine (2.5 eq) under argon with stirring. The solution was stirred at RT overnight. After completion of the reaction, 10 ml methanol was added to consume the excess of DMTrCl. The mixture was stirred for 5 min and the solvent was removed by rotary evaporation. The residue was dissolved in 50 ml ethyl acetate and the solution was washed with water and brine and dried over $MgSO_4$. After chromatography with ethyl acetate / hexanes (2/1), two diastereomers **107a** or **107b** and **108a** or **108b** were obtained in ~ 45% and ~ 40% yield respectively.

107a: R_f = 0.40 (Eluent: ethyl acetate / hexanes = 2 / 1);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.61 ppm;

^{11}B NMR (160.4 MHz, $CDCl_3$) δ - 44.81 ppm;

LRMS (FAB-NBA) m/e 1138 ($[M+H]^+$, 0.4);

^1H NMR (500 MHz, $CDCl_3$): δ 9.25 (b, 1H, NH), 9.21 (b, 1H, NH), 7.51 (d, J = 1.5 Hz, 1H, ^5H-6), 7.05 (d, J =1.0 Hz, 1H, ^3H-6), 7.65 - 6.83 (m, 23H, aromatic protons), 6.37 - 6.33 (m, 2H, ^5H-1', ^3H-1'), 5.10 (m, 1H, ^5H-3'), 4.34 - 4.31 (m, 1H, ^3H-3'), 4.15 (m, ^5H-4'), 4.07 (m, 1H, ^3H-4'), 3.94 - 3.91 (m, 2H, CH_2O), 3.90 - 3.85 (m, 1H, ^3H-5'), 3.78 (s, 6H, CH_3O x 2), 3.70 - 3.65 (m, 1H, ^3H-5''), 3.45 - 3.34 (AB, 2H, ^5H-5', ^5H-5''), 2.45 (t, J = 6.1 Hz, 2H, CH_2CN) 2.44 - 2.39 (m, 1H, ^5H-2'), 2.35 - 2.28 (m, 2H, ^5H-2'', ^3H-2'), 2.00 -1.94 (m, 1H, ^3H-2''), 1.85 (d, J = 1.0 Hz, 3H, $^3CH_3C$-5), 1.45 (d, J = 1.5 Hz, 3H, $^5CH_3C$-5), 1.08 (s, 9H, SiMe3), 0.42, 0.38(d, J = 18.55 Hz, 3H, BH_3);

^{13}C NMR (125.7 MHz, $CDCl_3$): 163.61 (^5C-4), 158.78 (^3C-4), 150.40 (^5C-2), 150.31 (^3C-2), 140.96, 135.71 (^3C-6), 135.67 (^5C-6), 135.40, 135.02, 134.90, 132.90, 132.63, 130.28, 130.21, 130.01, 128.06, 128.00, 127.26, 115.88 (CH_2CN), 113.36, 111.78 (^5C-5), 111.49 (^3C-5), 87.29 ($\underline{C}$-O-^3C-5'), 85.20 (^3C-1'), 84.66 (d, J = 6.4 Hz, ^5C-4'), 84.58 (d, J = 3.8 Hz, ^3C-4'), 84.24 (^5C-1'), 79.08 (^5C-3'), 72.79 (^3C-3'), 65.62 (d, J = 5.5 Hz, ^3C-5'), 63.17 (^5C-5'), 61.20 (CH_2O), 55.25 (OCH_3), 39.92 (^3C-2'), 39.06 (^5C-2'), 26.77 (SiC$\underline{Me}_3$), 19.42 (d, J = 6.4 Hz, $\underline{C}H_2CN$), 18.94 (Si$\underline{C}Me_3$), 12.42 ($^3\underline{C}H_3C$-5), 11.74 ($^5\underline{C}H_3C$-5);

108a: R_f = 0.25 (Eluent: ethyl acetate / hexanes = 2 / 1)

^{31}P NMR (202.3 MHz, $CDCl_3$): 117.78 ppm;

^{11}B NMR (160.4 MHz, $CDCl_3$): - 43.87 ppm;

LRMS (FAB-NBA) m/e: 1138 ($[M+H]^+$, 1.3);

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (b, 1H, NH), 8.62 (b, 1H, NH), 7.54 (d, J = 1.5 Hz, 1H, ^{5}H-6), 7.01 (d, J =1.0 Hz, 1H, ^{3}H-6), 7.63 - 6.83 (m, 23H, aromatic protons), 6.37 - 6.34 (dd, 1H, J = 5.4 Hz, J = 5.2 Hz, ^{5}H-1'), 6.34 - 6.32 (dd, 1H, J = 5.6 Hz, J = 6.1 Hz, ^{3}H-1'), 5.15 (m, 1H, ^{5}H-3'), 4.29 - 4.26 (m, 1H, ^{3}H-3'), 4.09 - 4.05 (m, 2H, CH_2O), 4.01 (m, 2H, ^{5}H-4', ^{3}H-4'), 3.86 - 3.82 (m, 1H, ^{3}H-5'), 3.79 (s, 6H, CH_3O x 2), 3.63 - 3.58 (m, 1H, ^{3}H-5''), 3.34 (m, 2H, ^{5}H-5', ^{5}H-5''), 2.65 - 2.62 (m, 2H, CH_2CN) 2.53 - 2.49 (m, 1H, ^{5}H-2'), 2.43 - 2.37 (m, 1H, ^{5}H-2'')2.31- 2.26 (m, 1H, ^{3}H-2'), 1.94 -1.88 (m, 1H, ^{3}H-2''), 1.86 (d, J = 1.0 Hz, 3H, $^3CH_3$C-5), 1.44 (d, J = 1.5 Hz, 3H, $^5CH_3$C-5), 1.06 (s, 9H, $SiMe_3$), 0.41, 0.37(d, J = 18.55 Hz, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H -5''), (^{3}H-5', ^{3}H-5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'').

107b: R_f = 0.39 (Eluent: ethyl acetate / hexanes = 2 / 1)

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.47 ppm;

^{11}B NMR (160.4 MHz, $CDCl_3$) δ - 44.23 ppm;

LRMS (FAB-NBA) m/e 1014 ($[M+H]^+$, 1.5);

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.28 (b, 1H, NH), 9.27 (b, 1H, NH), 7.52 (d, J = 1.5 Hz, 1H, ^{5}H-6), 7.13 (d, J =1.0 Hz, 1H, ^{3}H-6), 7.35 - 6.81 (m, 13H, aromatic protons), 6.40 - 6.37 (dd, J = 5.4 Hz, J = 5.6 Hz, 1H, ^{5}H-1'), 6.15 - 6.13 (dd, J = 6.6 Hz, J = 6.8 Hz, 1H, ^{3}H-1'), 5.18 (t, 1H, ^{5}H-3'), 4.41 - 4.38 (q, 1H, ^{3}H-3'), 4.25 - 4.17 (m, 3H, ^{5}H-4', ^{3}H-5', ^{3}H-5''), 4.12 - 4.07 (m, 2H, CH_2O), 3.97 (q, 1H, ^{3}H-4'), 3.77 (s, 6H, CH_3O x 2), 3.46 - 3.37 (AB, 2H, ^{5}H-5', ^{5}H-5''), 2.64 - 2.60 (m, 2H, CH_2CN), 2.58 - 2.56 (m, 1H, ^{5}H-2'), 2.41 - 2.35 (m, 1H, ^{5}H-2''), 2.25 (m, 2H, ^{3}H-2', ^{3}H-2''), 1.88 (d, J = 1.0

Hz, 3H, $^3CH_3C$-5), 1.44 (d, J = 1.5 Hz, 3H, $^5CH_3C$-5), 0.87 (s, 9H $SiCMe_3$), 0.071 (s, 6H, $SiMe_2$), 0.42 (b, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3C$-5), (^{3}H-6, $^3CH_3C$-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H -5''), (^{3}H-5', ^{3}H-5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.73 (^{5}C-4), 163.62 (^{3}C-4), 158.79, 150.48 (^{5}C-2), 150.24 (^{3}C-2), 143.99, 136.01 (^{3}C-6), 135.05 (^{5}C-6), 134.94, 134.91, 130.01, 128.08, 128.01, 127.26, 116.07 (CH_2CN), 113.37, 111.83 (^{5}C-5), 111.35 (^{3}C-5), 87.32 (<u>C</u>-O-^{3}C-5'), 85.98 (^{3}C-1'), 84.69 (d, J = 4.6 Hz, ^{5}C-4'), 84.56 (d, J = 6.4 Hz, ^{3}C-4'), 84.35 (^{5}C-1'), 79.12 (^{5}C-3'), 71.32 (^{3}C-3'), 65.69 (d, J = 5.5 Hz, ^{3}C-5'), 63.22 (^{5}C-5'), 61.41 (CH_2O), 55.26 (OCH_3), 40.03 (^{3}C-2'), 39.22 (^{5}C-2'), 25.65 (Si<u>C</u>Me_3), 19.61 (d, J = 5.5 Hz, <u>C</u>H_2CN), 17.87 (Si<u>C</u>Me_3), 12.49 (^{3}<u>C</u>H_3C-5), 11.76 (^{5}<u>C</u>H_3C-5), -4.67, -4.86 (Si<u>Me</u>$_2$);

HMQC (500 MHz, $CDCl_3$) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (C<u>H</u>$_2$O, <u>C</u>H_2O), (OC<u>H</u>$_3$, O<u>C</u>H_3), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (C<u>H</u>$_2$CN, <u>C</u>H_2CN), (^{3}C<u>H</u>$_3$C-5, ^{3}<u>C</u>H_3C-5), (^{5}C<u>H</u>$_3$C-5, ^{5}<u>C</u>H_3C-5), (SiC<u>Me</u>$_3$, SiC<u>Me</u>$_3$).

108b: R_f = 0.25 (Eluent: ethyl acetate / hexanes = 2 / 1);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.43 ppm;

^{11}B NMR (160.4 MHz, $CDCl_3$) δ - 44.80 ppm;

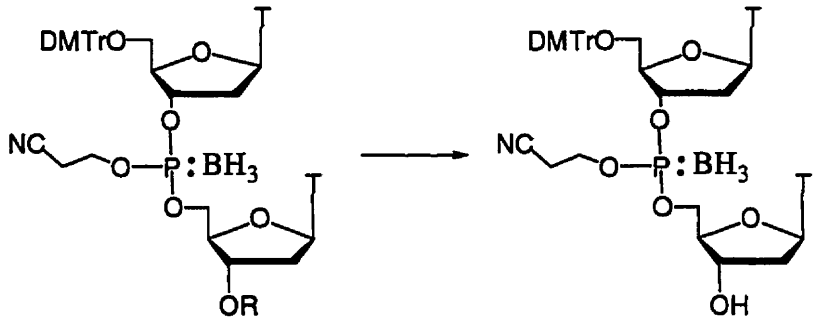
LRMS (FAB-NBA) m/e 1014 ($[M+H]^+$, 0.8);

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (b, 1H, NH), 9.11 (b, 1H, NH), 7.53 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.11 (d, J =1.2 Hz, 1H, ^{3}H-6), 7.36 - 6.81 (m, 13H, aromatic protons), 6.41 - 6.38 (dd, J = 5.4 Hz, J = 5.4 Hz, 1H, ^{5}H-1'), 6.4 (t, J = 6.6 Hz, 1H, ^{3}H-1'), 5.20 (t, 1H, ^{5}H-3'), 4.36 - 4.33 (m, 1H, ^{3}H-3'), 4.21 - 4.19 (m, 3H, ^{5}H-4'), 4.20 - 4.16 (m, 2H, CH_2O), 4.19 (^{3}H-5', ^{3}H-5''), 3.94 (q, 1H, ^{3}H-4'), 3.77 (s, 6H, CH_3O x 2), 3.43 - 3.37 (m, 2H, ^{5}H-5', ^{5}H-5''), 2.72 (m, 2H, CH_2CN), 2.61 - 2.57 (m, 1H, ^{5}H-2'), 2.46 - 2.40 (m, 1H, ^{5}H-2''), 2.25 - 2.16 (m, 2H, ^{3}H-2', ^{3}H-2''), 1.88 (d, J = 1.2 Hz, 3H, $^{3}CH_3$C-5), 1.43 (d, J = 1.2 Hz, 3H, $^{5}CH_3$C-5), 0.86 (s, 9H $SiCMe_3$), 0.049 (s, 6H, $SiMe_2$), 0.44 (b, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^{5}CH_3$C-5), (^{3}H-6, $^{3}CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H -5''), (^{3}H-5', ^{3}H-5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.69 (^{5}C-4), 163.61 (^{3}C-4), 158.80, 158.78, 150.58 (^{5}C-2), 150.19 (^{3}C-2), 144.01, 135.83 (^{3}C-6), 135.07 (^{5}C-6), 134.97, 134.94, 130.00, 128.09, 127.99, 127.24, 116.28 (CH_2**C**N), 113.39, 111.83 (^{5}C-5), 111.45 (^{3}C-5), 87.35 (**C**-O-^{3}C-5'), 85.74 (^{3}C-1'), 84.84 (d, J = 4.6 Hz, ^{5}C-4'), 84.60 (d, J = 6.4 Hz, ^{3}C-4'), 84.40 (^{5}C-1'), 79.30 (^{5}C-3'), 71.30 (^{3}C-3'), 65.80 (d, J = 5.5 Hz, ^{3}C-5'), 63.33 (^{5}C-5'), 61.59 (CH_2O), 55.26 (OCH_3), 40.07 (^{3}C-2'), 39.41 (d, J = 3.2 Hz, ^{5}C-2'), 25.64 (SiC**Me**$_3$), 19.74 (d, J = 5.5 Hz, **C**H_2CN), 17.86 (Si**C**Me_3), 12.47 (^{3}**C**H_3C-5), 11.74 (^{5}**C**H_3C-5), -4.68, -4.87 (Si**Me**$_2$).

Dimers Sp-109 and Rp-110.



107a or **108a**, R = TBDPS
107b or **108a**, R = TBDMS

Sp-**109** or Rp-**110**

To a solution of **107a** (0.1 mmol) (or **107b**, **108a**, **108b**) in 5 ml THF, HOAc (24 eq) was added, followed by TBAF (3 eq) was added. The reaction mixture was stirred at RT for 4 h. After completion of the reaction, 10 ml water was added to quench the reaction. The mixture was stirred for 5 min and the solvent was removed by rotary evaporation. The residue was dissolved in 50 ml ethyl acetate and the solution was washed with water and brine and dried over $MgSO_4$. After chromatography with ethyl acetate / methanol (10/1) Sp-**109** or Rp-**110** was obtained in the yield of ~ 60% and ~ 70%, respectively.

Sp-**109**: R_f = 0.38 (eluent: ethyl acetate / methanol = 10/1)

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 118.02 ppm;

^{11}B NMR (96.2 MHz, $CDCl_3$) δ - 44.01 ppm;

LRMS (FAB, NBA) m/e 900 $[M+H]^+$;

^1H NMR (500 MHz, $CDCl_3$) δ 10.08 (b, NH), 9.82 (b, NH), 7.54 (s, 1H, ^{5}H-6), 7.21 (s 1H, ^{3}H-6), 7.35 - 6.81 (m, 13H, aromatic protons), 6.36 - 6.33 (dd, 1H, J = 5.1 Hz, J = 5.4 Hz, ^{5}H-1'), 6.25 - 6.22 (dd, 1H, J = 6.6 Hz, J = 6.8 Hz, ^{3}H-1'), 5.16 (m, 1H, ^{5}H-3'), 4.47 (m, 1H, ^{3}H-3'), 4.28 (m, 2H, ^{3}H-5', ^{3}H-5''), 4.23 (m, 1H, ^{5}H-4'), 4.21 - 4.12 (m, 2H, CH_2O), 4.10 (m, 1H, ^{3}H-4'), 3.76 (s, 6H, CH_3O x 2), 3.45 - 3.37 (AB, 2H, ^{5}H-5', ^{5}H-5''), 2.68 (m, 3H, CH_2CN, ^{5}H-2'), 2.41 - 2.34 (m, 2H, ^{5}H-2'', ^{3}H-2'), 2.25 - 2.20 (m, 1H, ^{3}H-2''), 1.88 (s, 3H, $^3CH_3$C-5), 1.46 (s, 3H, $^5CH_3$C-5), 0.40 (b, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.10 (^{5}C-4), 163.90 (^{3}C-4), 151.04 (^{5}C-2), 150.68 (^{3}C-2), 135.88 (^{3}C-6), 134.96 (^{5}C-6), 158.76, 144.00, 130.02, 128.09, 127.96, 127.26, 113.39(aromatic carbons), 116.51 (CH_2CN), 111.99 (^{5}C-5), 111.43 (^{3}C-5), 87.34, 85.48 (^{3}C-1'), 84.72 (d, J = 5.4 Hz, ^{5}C-4'), 84.54 (^{5}C-1'), 84.30 (d, J = 6.4 Hz, ^{3}C-4'), 79.56 (^{5}C-3'), 70.91 (^{3}C-3'), 66.12 (d, J = 4.6 Hz, ^{3}C-5'), 63.27 (^{5}C-5'), 61.52 (CH_2O), 55.27 (OCH_3), 39.62 (^{3}C-2'), 39.33 (^{5}C-2'), 19.65 (d, J = 6.4 Hz, CH_2CN), 12.50 ($^3CH_3$C-5), 11.84 ($^5CH_3$C-5);

HMQC (500 MHz, $CDCl_3$) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), ($\mathbf{CH_2}O$, $\mathbf{C}H_2O$), ($O\mathbf{CH_3}$, $O\mathbf{C}H_3$), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), ($\mathbf{CH_2}CN$, $\mathbf{C}H_2CN$), ($^3\mathbf{CH_3}$C-5, $^3\mathbf{C}H_3$C-5), ($^5\mathbf{CH_3}$C-5, $^5\mathbf{C}H_3$C-5).

Rp-**110**: R_f = 0.36 (eluent: ethyl acetate / methanol = 10/1);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.01 ppm;

^{11}B NMR (96.2 MHz, $CDCl_3$) δ -44.36 ppm;

LRMS (FAB, NBA) m/e 900 $[M+H]^+$;

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (b, NH), 9.75 (b, NH), 7.52 (s, 1H, ^{5}H-6), 7.18 (s 1H, ^{3}H-6), 7.35 - 6.81 (m, 13H, aromatic protons), 6.38 - 6.35 (dd, 1H, J = 5.4 Hz, J = 5.4 Hz, ^{5}H-1'), 6.23 - 6.21 (dd, 1H, J = 6.6 Hz, J = 6.6 Hz, ^{3}H-1'), 5.13 (m, 1H, ^{5}H-3'), 4.42 (m, 1H, ^{3}H-3'), 4.25 (m, 3H, ^{5}H-4', ^{3}H-5', ^{3}H-5''), 4.18 (m, 2H, CH_2O), 4.05 (m, 1H, ^{3}H-4'), 3.75 (s, 6H, CH_3O x 2), 3.41 (m, 2H, ^{5}H-5', ^{5}H-5''), 2.72 (t, 2H,

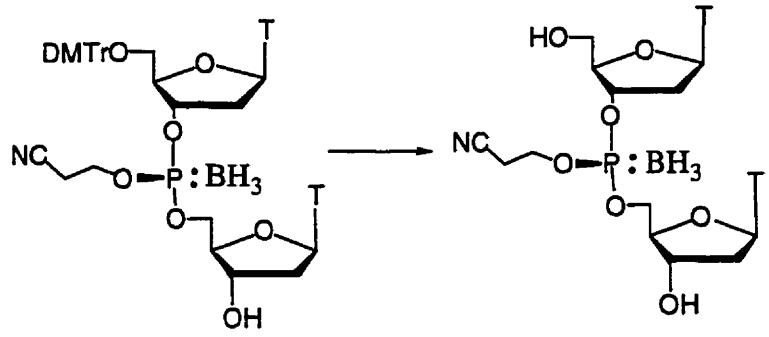
CH_2CN), 2.61 - 2.58 (m, 1H, ^{5}H-2'), 2.45 - 2.40 (m, 1H, ^{5}H-2''), 2.37 -2.32 (m, 1H, ^{3}H-2'), 2.21 - 2.15 (m, 1H, ^{3}H-2''), 1.86 (s, 3H, $^3CH_3$C-5), 1.44 (s, 3H, $^5CH_3$C-5), 0.41 (b, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.08 (^{5}C-4), 163.88 (^{3}C-4), 151.06 (^{5}C-2), 150.64 (^{3}C-2), 135.78 (^{3}C-6), 135.03 (^{5}C-6), 158.75, 158.74, 144.05, 130.05, 128.09, 127.97, 127.24, 113.39,(aromatic carbons), 116.61(CH_2**C**N), 112.02 (^{5}C-5), 111.51 (^{3}C-5), 87.33, 85.42 (^{3}C-1'), 84.72 (d, J = 3.6 Hz, ^{5}C-4'), 84.48 (^{5}C-1'), 84.35 (d, J = 6.4 Hz, ^{3}C-4'), 79.29 (^{5}C-3'), 70.88 (^{3}C-3'), 66.15 (d, J = 4.6 Hz, ^{3}C-5'), 63.32 (^{5}C-5'), 61.73 (CH_2O), 55.28 (OCH_3), 39.63 (^{3}C-2'), 39.23 (^{5}C-2'), 19.73 (d, J = 5.5 Hz, **C**H_2CN), 12.49 (^{3}**C**H_3C-5), 11.81 (^{5}**C**H_3C-5);

HMQC (500 MHz, $CDCl_3$) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (**C**H_2O, **C**H_2O), (O**C**H_3, O**C**H_3), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (**C**H_2CN, **C**H_2CN), (^{3}**C**H_3C-5, ^{3}**C**H_3C-5), (^{5}**C**H_3C-5, ^{5}**C**H_3C-5).

Dithymidine Boranophosphate Cyanoethyl Esters (Sp-113 and Rp-114).



Sp-**109** or Rp-**110** Sp-**113** or Rp-**114**

A solution of Sp-**109** (33 mg) or Rp-**110** (32 mg) in 3 ml 70% acetic acid - water was stirred until the removal of dimethoxytrityl group was complete (0.5 h). The solvent was evaporated and coevaporated with methanol twice. After chromatography with ethyl acetate/methanol (1/0 - 10/1), Sp-**113** or Rp-**114** was obtained in 87% and 90% yield, respectively.

Sp-**113**: ^{31}P NMR (202.3 MHz, CD_3OD) δ 117.75 ppm;

^{11}B NMR (96.2 MHz, CD_3OD) δ -43.98 ppm;

LRMS (FAB, NBA) m/e 598 $[M+H]^+$;

^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.47 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.31 - 6.26 (m, 2H, ^{5}H-1', ^{3}H-1'), 5.14 - 5.11 (m, 1H, ^{5}H-3'), 4.43 - 4.40 (m, 1H, ^{3}H-3'), 4.34 - 4.31 (m, 2H, CH_2O), 4.30 - 4.25 (m, 2H, ^{3}H-5', ^{3}H-5''), 4.20 (m, 1H, ^{5}H-4'), 4.05 - 4.03 (m, 1H, ^{3}H-4'), 3.78 (m, 2H, ^{5}H-5', ^{5}H-5''), 2.89 (t, 2H, CH_2CN), 2.51 - 2.46 (m, 1H, ^{5}H-2'), 2.38 - 2.32 (m, 1H, ^{5}H-2''), 2.28 - 2.26 (m, 2H, ^{3}H-2', ^{3}H-2''), 1.91 (d, J = 1 Hz, 3H, $^3CH_3$C-5), 1.87 (d, J = 1 Hz, 3H, $^5CH_3$C-5), 0.42 (b, 3H, BH_3);

COSY (500 MHz, CD_3OD) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'),

(^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5''), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, CD_3OD) δ 166.31 (^{5}C-4, ^{3}C-4), 152.35 (^{5}C-2), 152.27 (^{3}C-2), 137.81 (^{5}C-6), 137.65 (^{3}C-6), 118.57 (CH_2CN), 112.07 (^{5}C-5), 111.93 (^{3}C-5) , 87.32 (d, J = 4.6 Hz, ^{5}C-4'), 86.37 (^{5}C-1'), 86.09 (^{3}C-1'), 85.94 (d, J = 6.4 Hz, ^{3}C-4'), 80.12 (^{5}C-3'), 71.74 (^{3}C-3'), 67.48 (d, J = 3.2 Hz, CH_2O), 63.29 (^{5}C-5'), 62.59 (^{3}C-5'), 40.42 (^{3}C-2'), 39.84 (^{5}C-2'), 20.23 (d, J = 6.4 Hz, CH_2CN), 12.65 ($^3CH_3$C-5), 11.48 ($^5CH_3$C-5);

HMQC (500 MHz, CD_3OD) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), ($C\mathbf{H}_2O$, $\mathbf{C}H_2O$), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), ($C\mathbf{H}_2CN$, $\mathbf{C}H_2CN$), ($^3C\mathbf{H}_3$C-5, $^3\mathbf{C}H_3$C-5), ($^5C\mathbf{H}_3$C-5, $^5\mathbf{C}H_3$C-5);

Rp-**114**: ^{31}P NMR (202.3 MHz, CD_3OD) δ 117.68 ppm;

^{11}B NMR (96.2 MHz, CD_3OD) δ -44.03 ppm;

LRMS (FAB, NBA) m/e 598 $[M+H]^+$;

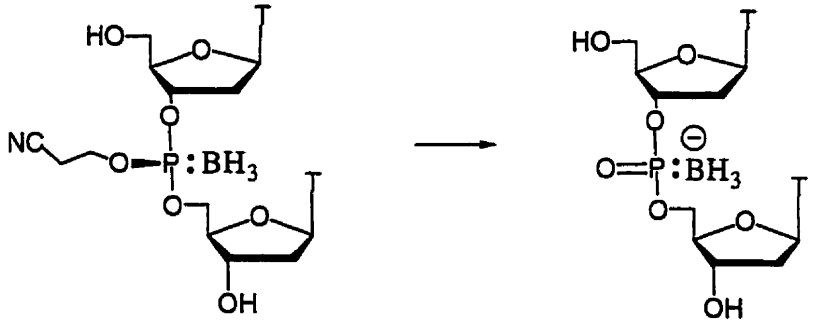
^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.47 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.31 - 6.26 (m, 2H, ^{5}H-1', ^{3}H-1'), 5.16 - 5.13 (m, 1H, ^{5}H-3'), 4.42 - 4.38 (m, 1H, ^{3}H-3'), 4.33 (m, 2H, CH_2O), 4.30 - 4.26 (m, 2H, ^{3}H-5', ^{3}H-5''), 4.21 - 4.19 (m, 1H, ^{5}H-4'), 4.06 - 4.03 (m, 1H, ^{3}H-4'), 3.78 (m, 2H, ^{5}H-5', ^{5}H-5''), 2.89 (t, 2H, CH_2CN), 2.53 - 2.49 (m, 1H, ^{5}H-2'), 2.40 - 2.36 (m, 1H, ^{5}H-2''), 2.28 - 2.22 (m, 2H, ^{3}H-2', ^{3}H-2''), 1.90 (d, J = 1 Hz, 3H, $^3CH_3$C-5), 1.87 (d, J = 1 Hz, 3H, $^5CH_3$C-5), 0.41 (b, 3H, BH_3);

COSY (500 MHz, CD_3OD) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (CH_2O, CH_2CN), (^{3}H-4', ^{3}H-5''), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, CD_3OD) δ 166.29 (^{5}C-4, ^{3}C-4), 152.36 (^{5}C-2), 152.26 (^{3}C-2), 137.81 (^{5}C-6), 137.67 (^{3}C-6), 118.57 (CH_2**C**N), 112.07 (^{5}C-5), 111.93 (^{3}C-5) , 87.32 (d, J = 4.6 Hz, ^{5}C-4'), 86.43 (^{5}C-1'), 86.15 (^{3}C-1'), 85.98 (d, J = 6.4 Hz, ^{3}C-4'), 79.88 (d, J = 2.7 Hz, ^{5}C-3'), 71.78 (^{3}C-3'), 67.52 (d, J = 4.6 Hz, CH_2O), 63.38 (^{5}C-5'), 62.55 (^{3}C-5'), 40.41 (^{3}C-2'), 39.84 (d, J = 3.7 Hz, ^{5}C-2'), 20.22 (d, J = 6.4 Hz, **C**H_2CN), 12.63 (^{3}**C**H_3C-5), 11.48 (^{5}**C**H_3C-5);

HMQC (500 MHz, CD_3OD) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (**C**H_2O, **C**H_2O), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (**C**H_2CN, **C**H_2CN), (^{3}**C**H_3C-5, ^{3}**C**H_3C-5), (^{5}**C**H_3C-5, ^{5}**C**H_3C-5).

Dithymidine Boranophosphates (Sp-115 and Rp-116).



Sp-**113** or Rp-**114** Sp-**115** or Rp-**116**

A solution of Sp-**113** (10 mg) or Rp-**114** (17 mg) in 28% NH_4OH / methanol was stirred at RT overnight. The flask was cooled in ice and opened to the atmosphere. After

allowing the ammonia to escape, the solution was lyophilized to give a white solid or the solvent was removed in high vacuum rotary evaporator. The crude product can be washed with ethyl acetate to give pure title compound Sp-**115** or Rp-**116**, or purified by chromatography with ethyl acetate / methanol = 10/1 - 3/1. Yield: 80% for Sp-**115** and 75% for Rp-**116**.

Sp-**115**: ^{31}P NMR (202.3 MHz, D_2O) δ 93.51 ppm;

^{11}B NMR (96.2 MHz, D_2O) δ - 41.64 ppm;

ES(-) MS (TEA/H_2O) m/e 542.2 ([M-H]$^-$, 22.18), 543.2 (100), 544.1 (72.80), 545.2 (33.64), 546.2 (11.57);

^{1}H NMR (500 MHz, D_2O) δ 7.51 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.48 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.17 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{5}H-1'), 6.07 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{3}H-1'), 4.68 - 4.64 (m, 1H, ^{3}H-3'), 4.43 - 4.41 (m, 1H, ^{5}H-3'), 4.00 - 3.97 (m, 1H, ^{3}H-4', ^{5}H-4'), 3.94 - 3.92 (m, 2H, ^{5}H-5', ^{5}H-5''), 3.69 -3.59 (AB, 2H, ^{3}H-5', ^{3}H-5''), 2.36 - 2.31 (m, 1H, ^{3}H-2'), 2.22 (m, 2H, ^{5}H-2', ^{5}H-2''), 2.20 - 2.16 (m, 1H, ^{3}H-2''), 1.75 (d, J = 1 Hz, 3H, $^5CH_3$C-5), 1.71 (d, J = 1 Hz, 3H, $^3CH_3$C-5), 0.20 (b, 3H, BH_3);

COSY (500 MHz, D_2O) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-4', ^{3}H-5''), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, D_2O) δ 166.18 (^{5}C-4), 166.05 (^{3}C-4), 151.43 (^{5}C-2), 151.23(^{3}C-2), 137.07 (^{5}C-6, ^{3}C-6), 111.26 (^{5}C-5), 111.19 (^{3}C-5) , 85.56 (d, J = 4.6 Hz, ^{3}C-4'), 85.03 (d, J = 6.4 Hz, ^{5}C-4'), 84.88 (^{3}C-1'), 84.45 (^{5}C-1'), 72.61 (d, J = 3.0 Hz, ^{3}C-3'), 70.42 (^{5}C-3'), 61.37 (d, J = 4.6 Hz, ^{5}C-5'), 60.55 (^{3}C-5'), 38.41 (^{5}C-2'), 37.60 (^{3}C-2'), 11.50 (^{5}**C**H_3C-5), 11.29 (^{3}**C**H_3C-5);

HMQC (500 MHz, D_2O) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}C**H**$_3$C-5, ^{5}C**H**$_3$C-5); (^{3}C**H**$_3$C-5, ^{3}C**H**$_3$C-5).

Rp-**116**: ^{31}P NMR (202.3 MHz, D_2O) δ 93.76 ppm;

^{11}B NMR (96.2 MHz, D_2O) δ -41.29 ppm;

ES(-) MS (TEA/H_2O) m/e 542.1 ([M-H]$^-$, 25.34), 543.2 (100), 544.2(31.11), 545.1 (7.24);

^{1}H NMR (500 MHz, D_2O) δ 7.57 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.51 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.15 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{5}H-1'), 6.08 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{3}H-1'), 4.75 - 4.70 (m, 1H, ^{3}H-3'), 4.43 -4.40 (m, 1H, ^{5}H-3'), 4.02 (m, 1H, ^{3}H-4'), 4.00 (m, 1H, ^{5}H-4'), 3.98 -3.90 (m, 2H, ^{5}H-5', ^{5}H-5''), 3.70 - 3.61 (AB, 2H, ^{3}H-5', ^{3}H-5''), 2.38 - 2.34 (m, 1H, ^{3}H-2'), 2.24 -2.17 (m, 3H, ^{5}H-2', ^{5}H-2'', ^{3}H-2''), 1.76 (d, J = 1 Hz, 3H, ^{5}C**H**$_3$C-5), 1.73 (d, J = 1 Hz, 3H, ^{3}C**H**$_3$C-5), 0.20 (b, 3H, BH_3);

COSY (500 MHz, D_2O) (^{5}H-6, ^{5}C**H**$_3$C-5), (^{3}H-6, ^{3}C**H**$_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-4', ^{3}H-5''), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, D_2O) δ 166.14 (^{5}C-4), 166.06 (^{3}C-4), 151.36 (^{5}C-2), 151.24 (^{3}C-2), 137.09 (^{5}C-6), 137.04 (^{3}C-6), 111.19 (^{5}C-5), 111.15 (^{3}C-5), 85.55 (d, J = 4.6 Hz, ^{3}C-4'), 85.08 (d, J = 7.3 Hz, ^{5}C-4'), 84.87 (^{3}C-1'), 84.78 (^{5}C-1'), 72.04 (d, J = 4.6 Hz, ^{3}C-3'), 70.48 (^{3}C-3'), 61.44 (d, J = 3.7 Hz, ^{5}C-5'), 60.60 (^{3}C-5'), 38.61 (^{5}C-2'), 37.91 (^{3}C-2'), 11.42 (^{5}C**H**$_3$C-5), 11.30 (^{3}C**H**$_3$C-5);

HMQC (500 MHz, D_2O) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}C**H**$_3$C-5, ^{5}C**H**$_3$C-5); (^{3}C**H**$_3$C-5, ^{3}C**H**$_3$C-5).

3.5. References

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8. The sample was left in NMR tube several days, then ^{31}P NMR showed only one single peak at 28.5 ppm and the peaks at ~ 117 ppm and ~ 96 ppm disappeared.
9. Schinazi, R. F.; Lesnikowski, Z. J. *Nucleosides & Nucleotides* **1998**, *17*, 635.
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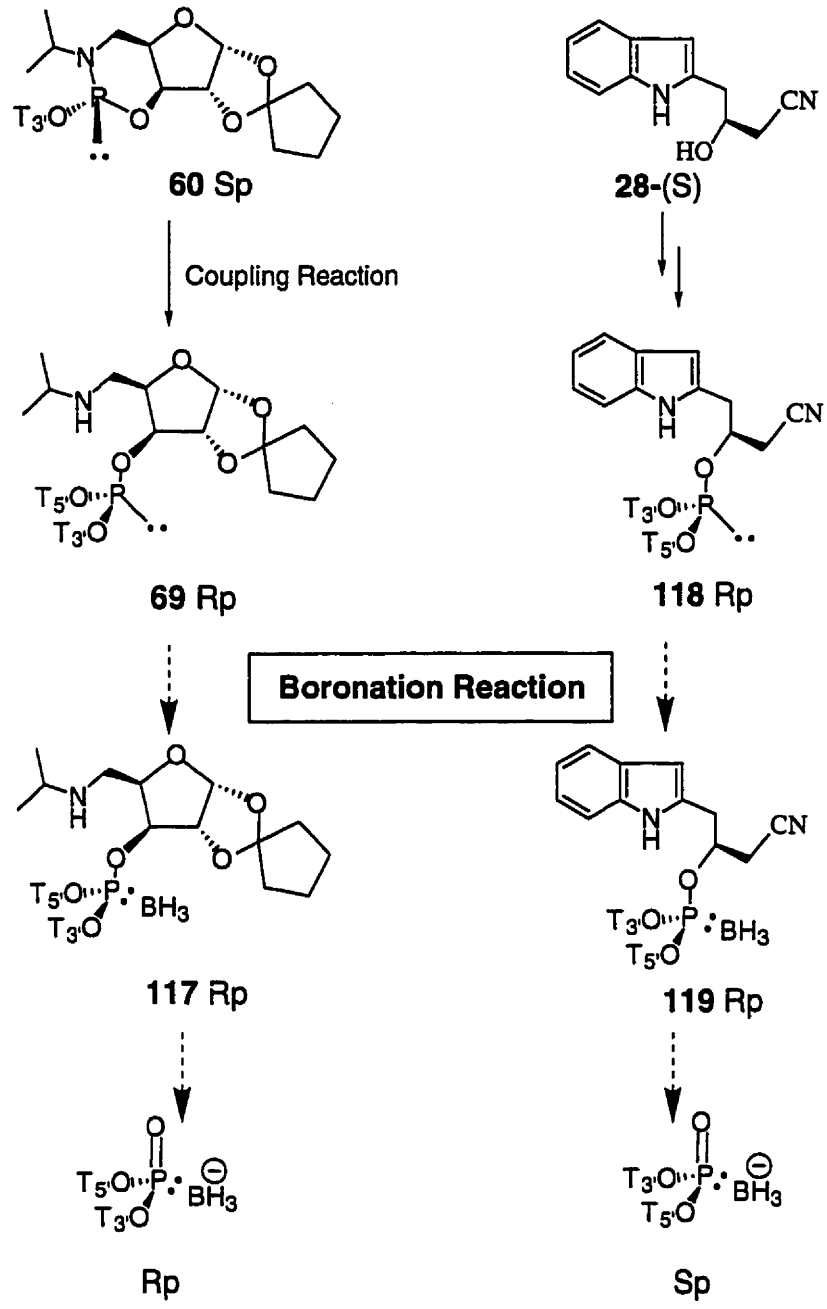
Chapter 4

A Stereoselective Synthesis of Dinucleotide Boranophosphates

4.1. Introduction

In Chapter 3, we described the synthesis of Sp and Rp diastereomers of dithymidine boranophosphate. Our main objective was to develop a method for the stereoselective synthesis of boranophosphates. For the nonstereoselective synthesis of boranophosphates, either the phosphoramidite or the H-phosphonate approach has been reported.[1] To the best of our knowledge, there is no literature report describing a chemical method for the stereoselective synthesis of boranophosphates.

In the stereoselective synthesis of phosphorothioates (Chapter 2), we have demonstrated that the stereochemistry of the coupling reaction between phosphoramidite **60** (Sp) and nucleoside ($\mathbf{T_5}$,**OH**) can be controlled to form stereoselectively phosphite triester **69** (Rp). In our research group, Wang[2] has also demonstrated that using indole-derivatives **28**-(S) and (R) as chiral auxiliaries can lead to the stereoselective synthesis of phosphite triester **118** (Rp). Therefore, if we could demonstrate that the boronation reaction was stereoselective, the phosphoramidite or indole approach should be adaptable for the stereoselective synthesis of boranophosphates (Scheme 4.1).

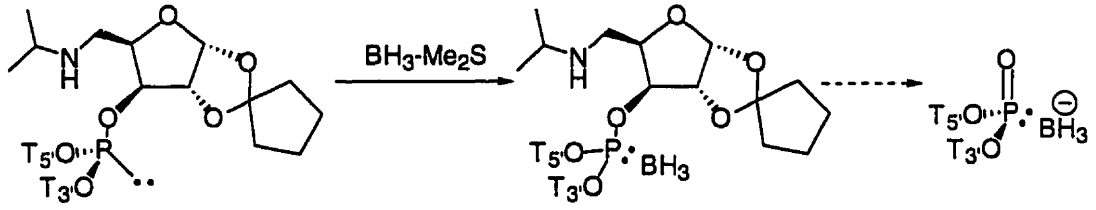


Scheme 4.1: The Possibility of the Stereoselective Synthesis of Boranophosphates.

4.2. Results and Discussion

4.2.1. The First Attempt Towards the Stereoselective Synthesis of Boranophosphates

As reported in Chapter 2, the phosphite triester **69** (Rp) was synthesized from phosphoramidite **60** (Sp). After reaction with dimethyl sulfide-borane (BH_3-Me_2S), the ^{31}P NMR showed that phosphite triester peak at 143.76 ppm disappeared within 5 - 10 min to give a broad peak at ~ 117 ppm for boranophosphate **117**, which did not allow the determination of the stereochemistry at phosphorus (Scheme 4.2).



69 Rp **117**

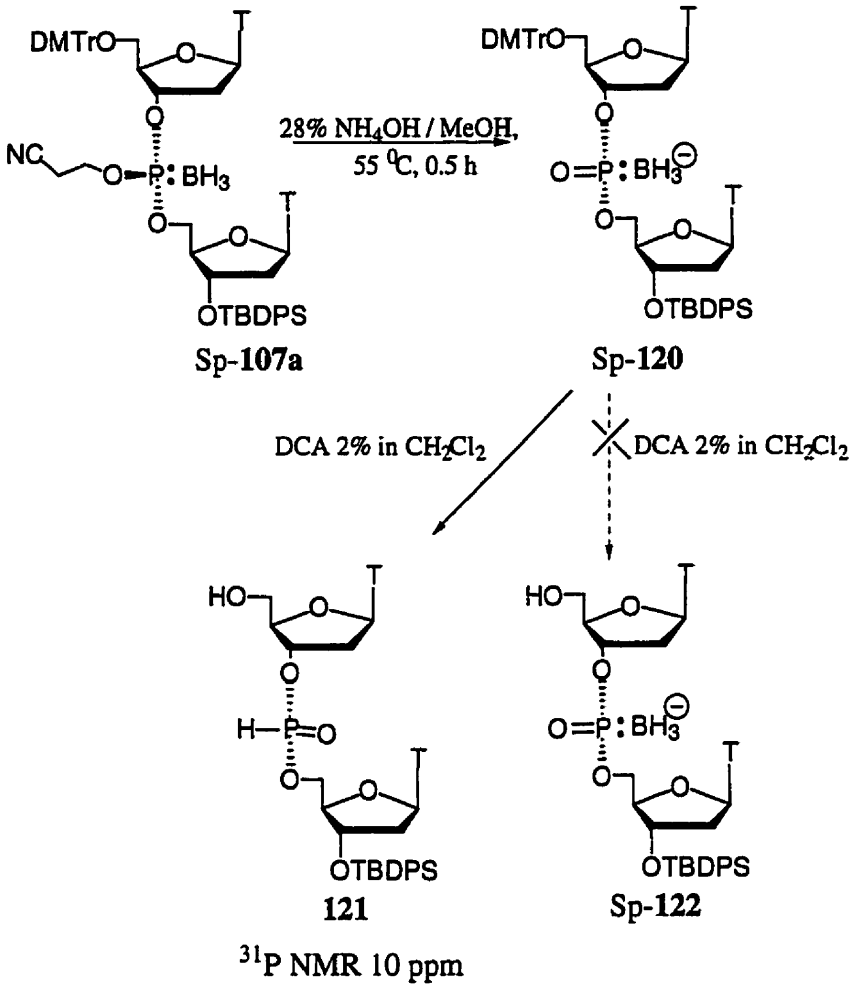
Scheme 4.2: The First Attempt Towards the Stereoselective Synthesis of Boranophosphates

The ^{31}P NMR spectra of boranophosphates are complicated due to direct bonding of phosphorus to the boron atom. Naturally abundant boron consists of 80.4% ^{11}B (spin I = 3/2) and 19.6% ^{10}B (spin I = 3). When a ^{31}P (I = 1/2) atom is bonded to a single ^{11}B, as in the -P-BH_3- linkage, four equally spaced and equal-intensity lines (a quartet with a 1-1-1-1 pattern) are expected in the ^{31}P NMR spectrum. If a ^{31}P is coupled to a ^{10}B atom, seven equally-spaced and equal-intensity lines (a septet with a 1-1-1-1-1-1-1 pattern) are expected. Thus, a P-B bond-containing sample with naturally abundant boron could be expected to show a complicated ^{31}P spectrum. In the experiment, we considered only the ^{11}B coupling and ignored the ^{10}B coupling in the ^{31}P spectrum.

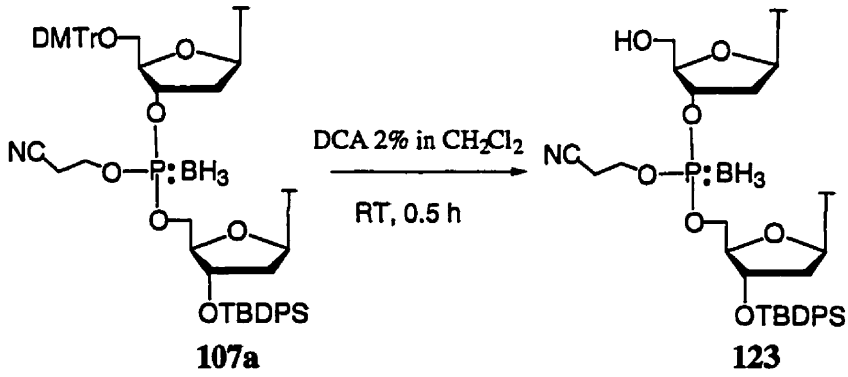
The stereoselectivity in the boronation step could not be derived from ^{11}B NMR spectrum either because of peak broadening. Although ^{11}B is a quadrupolar nucleus, line-broadening is not severe for observing the peak of the boranophosphate. Boron in the glass NMR tube and the NMR probe produce a broad background ^{11}B signal, which did not obstruct our observations.

In order to determine the stereoselectivity in the boronation step, we had to remove the chiral auxiliary to get the free boranophosphate dimers and then compared the ^{1}H NMR spectrum of the free boranophosphates as reported in Chapter 3. We have shown that the deprotection of the chiral auxiliary from protected phosphorothioate **62** (see Scheme 2.9) needed 70% TFA. It has been known that the -P-BH_3- linkage are stable at physiological conditions,[3] base condition, such as, NH_3-H_2O, and certain acid condition, such as acetic acid as discussed in Chapter 3. It is also reported that less than 10% of the phosphite-borane group is hydrolyzed to phosphate (by ^{11}B NMR and ^{31}P NMR) when dithymidine boranophosphate methyl ester **32** (see Scheme 1.10) is shaken at RT overnight in a mixture of 1N HCl and MeOH (1:1 v/v).[3] Before trying to remove the chiral auxiliary from boranophosphate **117** by using 70% TFA, we did the model reactions as shown in Scheme 4.3 to test the stability of -P-BH_3- linkage in acid condition.

The protected boranophosphate Sp-**107a** was synthesized as reported in Chapter 3. Deprotection of the cyanoethyl group with concentrated NH_4OH in methanol at RT for 0.5 h gave the boranophosphate Sp-**120**. Unfortunately, when we tried to remove the DMTr group at the 5'-hydroxyl position of Sp-**120** with 2% dichloroacetic acid in CH_2Cl_2, H-phosphonate **121** (^{31}P NMR 10 ppm, one single peak) was obtained instead of the expected dimer **122**. We therefore concluded that the boranophosphate linkage was not stable under strong acid condition. However, it should be noticed that the boranophosphate triester is stable in 2% dichloroacetic acid in CH_2Cl_2 (see Scheme 4.4), since the deprotected boranophosphate triester **123** was obtained from **107a**.



Scheme 4.3: The Model Reaction to Test the Stability of -P-BH_3- linkage in the Acid Condition

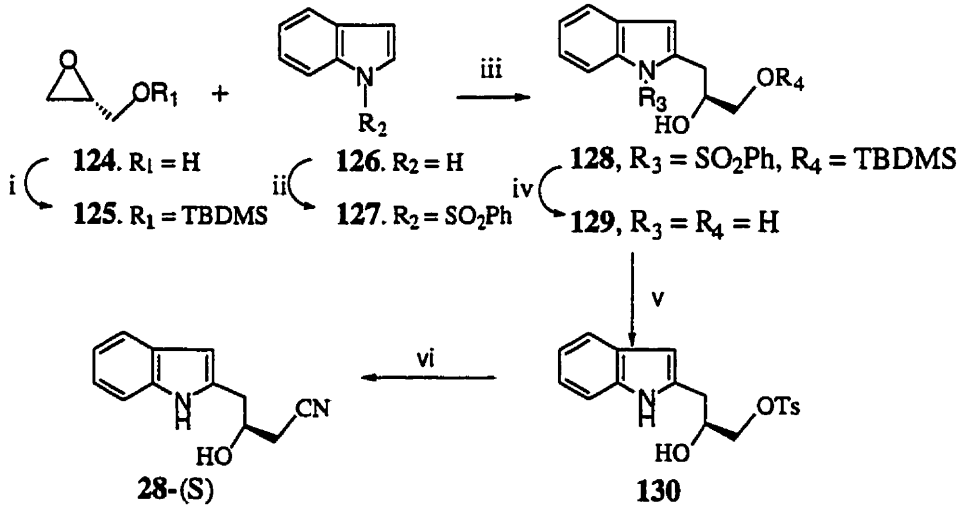


Scheme 4.4: The Stability of Boranophosphite Triester

In the indole-oxazaphosphorine approach to the stereoselective synthesis of phosphorothioates, the deprotection of the chiral auxiliary indole-derivative can be done by using base. We therefore investigated the use of chiral auxiliary **28** in the stereoselective synthesis of boranophosphates.

4.2.2. The Synthesis of Chiral Auxiliary

As reported,[2] the chiral auxiliary **28**-(S) was synthesized from (R)-glycidol **124**. The hydroxy group of (R)-glycidol **124** was protected with TBDMS to give **125**. The nitrogen of indole **126** was protected with phenylsulfonyl group to give **127**. Then the protected glycidol **125** was reacted with 1-phenylsulfonyl-2-lithioindole of **127** to give a diol **129**, after deprotection of **128** with KOH. The primary hydroxy group of diol **129** was transformed selectively to its tosylate **130**, which was then reacted with NaCN to form the desired chiral auxiliary **28**-(S) (Scheme 4.5).

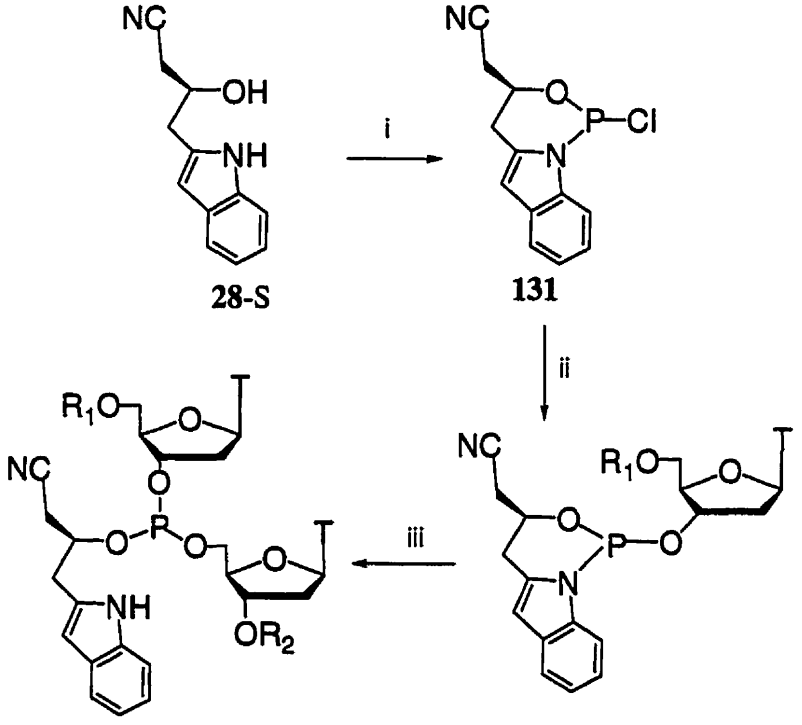


i. TBDMSCl, NEt_3, DMAP, CH_2Cl_2, 81%. ii. n-BuLi, THF, - 78 ^{0}C, then $PhSO_2Cl$, 90%.
iii. n-BuLi, -78 ^{0}C - 25 ^{0}C, overnight, 48%; iv. KOH, CH_3OH/H_2O (3:1), reflux, 87%.
v. TsCl (1.1 eq.), pyridine, 0 ^{0}C, overnight, 99%. vi. NaCN, DMF, 100^{0}C, 3 h, 64%.

Scheme 4.5: The Synthesis of Chiral Auxiliary

4.2.3. The Stereoselective Synthesis of Dinucleotide Boranophosphates

The reaction of **28**-(S) with PCl_3 in THF at 0 ^{0}C was completed in several minutes and gave phosphorochloridite **131** (^{31}P NMR 142.86 ppm); 5'-O-TBDMS-thymidine (**T_3,OH**) was then added at 0 ^{0}C to provide the indole-oxazaphosphorine **132a** consisting of two diastereomers in a ratio of 8 : 1 - 12 : 1 (^{31}P NMR 120.80, 120.98 ppm). When 3 eq. of **132a** was treated with 1 eq. of 3'-O-TBDPS-thymidine (**T_5,OH**), only one isomer **133a** (^{31}P NMR showed a single peak at 141.61 ppm) was obtained (Scheme 4.6).



133a, R_1 = TBDMS, R_2 = TBDPS
133b, R_1 = DMTr, R_2 = DMTr

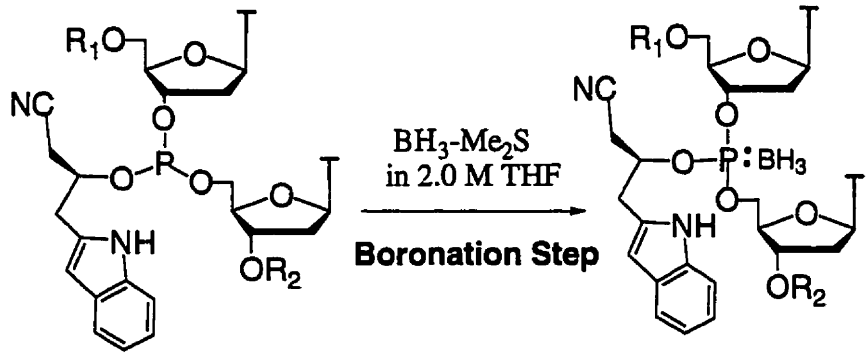
132a, R_1 = TBDMS
132b, R_1 = DMTr

i. PCl_3, Et_3N/THF, 0 ^{0}C; ii. **T_3,OH**/THF, 0 ^{0}C; iii. **T_5,OH**, DBU/THF, RT.

Scheme 4.6: The Stereoselective Synthesis of Phosphite Triesters

After reaction with dimethyl sulfide-borane (BH_3-Me_2S), the phosphite peak disappeared within 5 - 10 min to give a broad peak at 116.83 ppm for boranophosphate

134a (Scheme 4.7). The diastereomeric ratio could not be determined from ^{31}P NMR spectrum because of peak broadening.

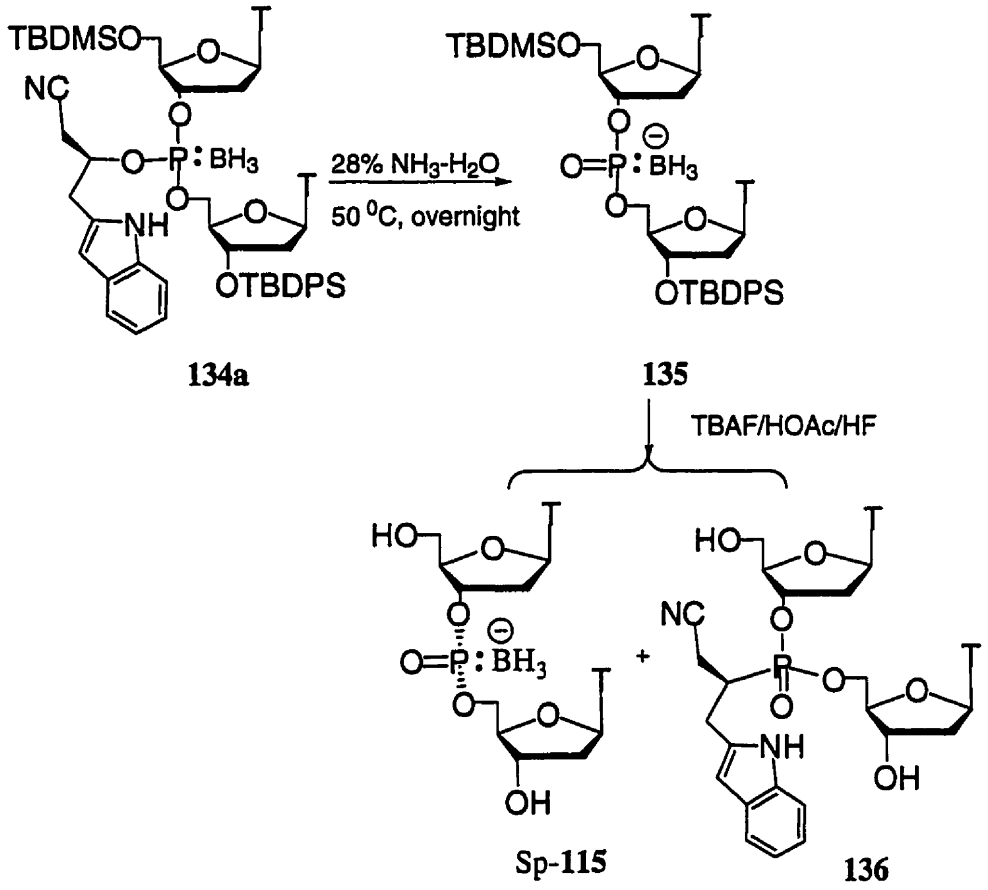


133a, R_1 = TBDMS, R_2 = TBDPS
133b, R_1 = DMTr, R_2 = DMTr

134a, R_1 = TBDMS, R_2 = TBDPS
134b, R_1 = DMTr, R_2 = DMTr

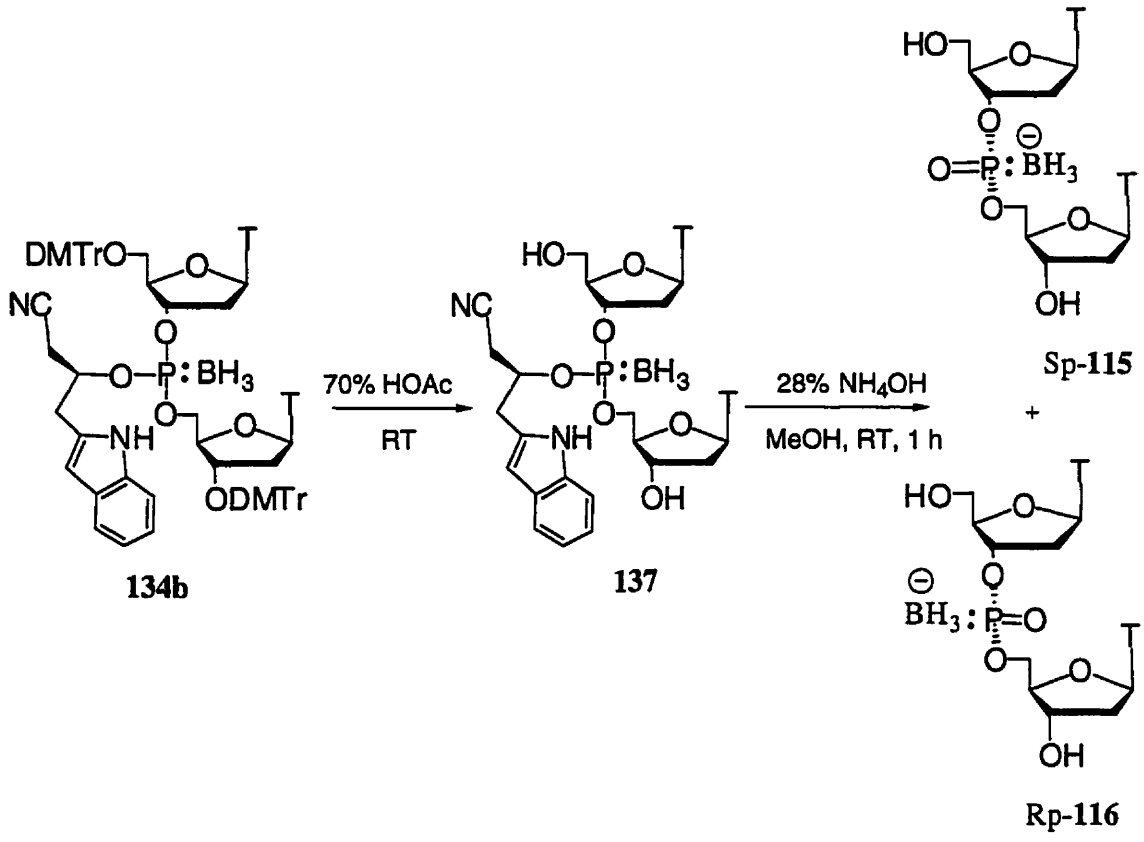
Scheme 4.7: The Boronation Reaction

As discussed in Section 4.2.1, the chiral auxiliary in **134a** was removed with 28% NH_4OH at 50 ^0C overnight to form the boranophospahte **135** (^{31}P NMR 96.53 ppm, broad). Without isolation, the crude product **135** was treated with TBAF or TBAF/HOAc/THF provided the boranophosphate Sp-**115** (^{31}P NMR ~ 93 ppm, broad) in low yield and another product showed a single peak at ~ 29 ppm in the ^{31}P NMR spectrum. The by-product had no BH_3 moiety since no peak was observed in the ^{11}B NMR spectrum. It was probably the alkylphosphonate **136** (Scheme 4.8).



Scheme 4.8: The Deprotection of Boranophosphate **134a**

In order to improve the yield of dinucleoside boranophosphate Sp-**115** and avoid the loss of the BH_3 moiety which was probably due to reaction with the fluoride ion, we repeated the reaction of phosphorochloridite **131** with 5'-O-dimethoxytrityl thymidine (**T_3,OH**). The indole-oxazaphosphorine **132b** was obtained in a diastereomeric ratio of 16 : 1. After adding 3'-O-dimethoxytrityl thymidine (**T_5,OH**), phosphite triester **133b** was obtained (Scheme 4.6). The reaction of **133b** with BH_3-Me_2S in 2.0 M THF gave boranophosphate **134b** (^{31}P NMR: 117.06 ppm, broad) (Scheme 4.7). Interestingly, the DMTr groups on **134b** were not removed in the boronation step even though the reaction was allowed to proceed overnight at RT (see Chapter 3).



134b 137

Scheme 4.9: The Stereoselective Synthesis of Dinucleotide Boranophosphate Sp-**115**

Reaction with 70% HOAc removed the DMTr groups and gave **135** which was converted to final compound Sp-**115**/Rp-**116** by using 28% NH_4OH in methanol at RT for 1 h (Scheme 4.9). In the coupling step (see Scheme 4.6), when 1 eq. of **132b** and 1 eq. of $\mathbf{T_5}$,**OH** were used, a 10 : 1 ratio of two diastereomers of phosphite triester **133b** (^{31}P NMR 140.95 : 140.86 ppm = 10 : 1) was obtained. After removing chiral auxiliary, the final compounds Sp-**115** and Rp-**116** were obtained in the same diastereomeric ratio of 10 : 1 as for **133b**. When 3 eq. of **132b** and 1 eq. of $\mathbf{T_5}$,**OH** were used, the coupling reaction provided only one diastereomer of phosphite triester **133b** (^{31}P NMR: 140.96 ppm) which was transformed to only one diastereomer of Sp-**115**. The diastereomeric ratio of Sp-**115** and Rp-**116** could not be obtained from ^{31}P NMR spectrum because of a broad peak at ~ 93 ppm. However, it was obtained from ^{1}H NMR with comparison with

the data in the literature (Figure 4.1). Also the absolute configurations at phosphorus in Sp-**115** and Rp-**116** were assigned by ^{1}H NMR comparison with literature data.[4]

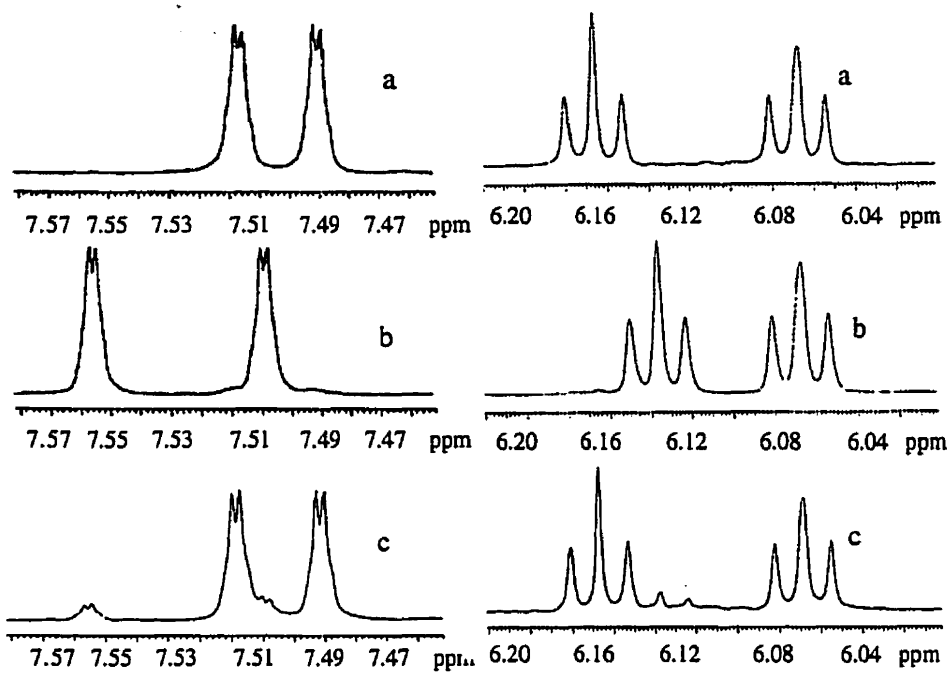


Figure 4.1: Part of ^{1}H NMR Spectra of Sp-**115** (a) and Rp-**116** (See Chapter 3) (b) and a diastereomer mixture of Sp-**115** and Rp-**116** (10 : 1) (c) in D_2O. Left spectra: assigned to ^{5}H-6 and ^{3}H-6, Right spectra: assigned to ^{5}H-1' and ^{3}H-1'.

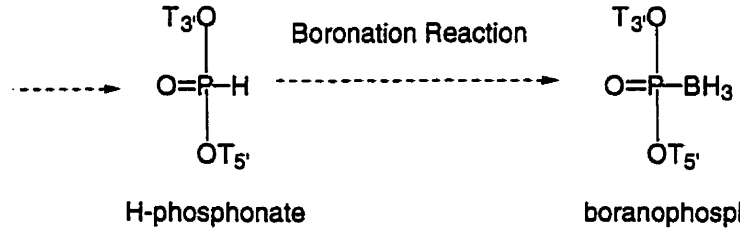
It is known that the Rp-dithymidine phosphorothioate can be synthesized from chiral auxiliary **28**-(S)[2] and that the conversion of phosphite triester to phosphorothioate proceeds with retention of stereochemistry.[5] Since Sp dithymidine boranophosphate was obtained from **28**-(S), it was concluded that the conversion of phosphite triester to boranophosphate by dimethyl sulfide - borane took place with retention of configuration. Noting that sulfur is the largest atom around the phosphorus center in a nucleotide phosphorothioate while boron is the smallest atom around the phosphorus center in a nucleotide boranophosphate, a Rp configuration in phosphorothioate corresponds to an Sp configuration in boranophosphate.

4.3. Conclusion

We have shown that a pure Sp dithymidine boranophosphate Sp-**115** can be synthesized from chiral auxiliary **28**-(S). It should be possible to get a pure Rp dithymidine boranophosphate from chiral auxiliary **28**-(R), since the Sp dithymidine phosphorothioate can be obtained from chiral auxiliary **28**-(R).[2] We have also proved that the boronation reaction with dimethyl sulfide-borane is a stereoretentive process. The method reported here might be adaptable to the solid phase synthesis of chiral oligonucleotide boranophosphates.

4.4. Future Outlook

We have shown that the phosphoramidite approach can lead to the stereoselective synthesis of boranophosphates. The H-phosphonate approach may also lead to the stereoselective synthesis of boranophosphates. To modify the H-phosphonate approach to the stereoselective synthesis of boranophosphates, two steps have to be done. First, a method leading to stereoselective synthesis of H-phosphonate has to be developed. Then, the boronation step of H-phosphonate has to be addressed (Scheme 4.10).



Scheme 4.10: The Possibility of the H-phosphonate Approach to the Stereoselective Synthesis of Boranophosphates.

Moreover, it is possible to develop a method for the stereoselective synthesis of alkylphosphonates or H-phosphonates from boranophosphates, since the borane group in boranophosphates seemed a good leaving group in the presence of the fluoride ion or strong acid (2% DCA) as shown in Scheme 3.3 (see Chapter 3) and Scheme 4.3 (see Chapter 4).

4.5. Experimental

General Materials and Methods

^{1}H NMR spectra were recorded on JOEL Eclipse 270, Varian UNITY 500 Spectrometers at 270 MHz, 500 MHz. ^{13}C NMR spectra were determined at 67.9 MHz, 125.7 MHz on JOEL Eclipse 270, Varian UNITY 500 spectrometer and ^{1}H and ^{13}C NMR chemical shifts are expressed in ppm relative to the internal tetramethylsilane (TMS). ^{31}P NMR spectra were taken on a Varian XL 300, UNITY 500 spectrometers at 121 and 202 MHz with proton-decoupling ({1H}). Positive ^{31}P NMR chemical shifts are expressed in ppm downfield from external 85% H_3PO_4. ^{11}B NMR spectra were recorded on Varian XL 300, UNITY 500 spectrometers at 96.2, 160.4 MHz. ^{11}B NMR chemical shifts were referenced externally to a solution of diethyletherboron trifluoride Et_2O-BF_3. Spin multiplicites are given with the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad peak.

Low resolution mass spectra were recorded on a KRATOS MS 25RFA mass spectrometer in the direct-inlet mode. High resolution mass spectra were performed on a ZAB 2F HS mass spectrometer. Negative electrospray mass spectrometry was performed on the Quattro II triple quadruple mass spectrometer in negative mode.

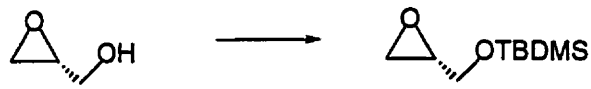
Melting points (m.p.) were determined on a Gallenkamp block and are uncorrected.

Dichloromethane was distilled from P_2O_5, THF from sodium benzophenone ketyl, triethylamine and acetonitrile were distilled from CaH_2. Pyridine was refluxed for 4 h with fine BaO and distilled over granular BaO under N_2. Anhydrous DMF was purchased from Aldrich in sure-seal bottles and used with no further drying. 3'-O-TBDPS-thymidine was given by Isis Pharmaceuticals. All other chemicals were purchased from Aldrich Chemical Company Inc. and were used without further purification.

Thin-layer chromatography (TLC) was performed using Kieselgel 60 F_{254} aluminum backed plates (0.2 mm thickness). Column chromatography was performed on 230 - 400 mesh silica gel (Merck).

All air sensitive experiments were performed under dry argon with freshly distilled anhydrous solvents and glassware previously dried overnight on an oven.

(S)-glycidyl *tert*-butyldimethylsilyl ether (125).



124 **125**

To a solution of (R)-glycidol **124** (5 g, 67.5 mmol) in dry dichloromethane (40 ml) containing triethylamine (10.3 ml, 74 mmol) was added a solution of TBDMSCl (11.2 g, 74 mmol) in dry dichloromethane (30 ml) at 0 ^{0}C and DMAP (0.33 g, 2.7 mmol). The mixture was allowed to warm up to room temperature and stirred for 5 h. Then triethylammonium chloride was filtered off and washed with dichloromethane (2 x 10 ml). The filtrate was washed with brine (2 x 50 ml), dried over anhydrous $MgSO_4$ and was concentrated on a rotary evaporator. The resulting solution was passed through a short silica gel column to remove polar impurities and then eluted with hexane/ethyl acetate (3 : 2). After removing the solvent, a light yellow oil was collected and distilled under vacuum (50 - 56 ^{0}C/4.5 mmHg) to provide pure (S)-glycidyl *tert*-butyldimethylsilyl ether **125** (10.3 g, 81%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.81 - 3.61 (m, 2H, CH_2OSi), 3.04 (m,

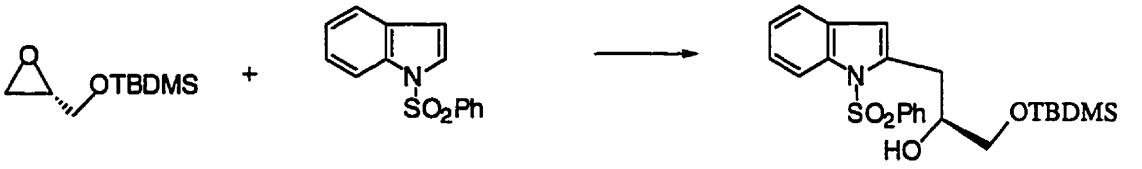
1H, CH), 2.72 - 2.59 (m, 2H, CH_2O), 0.86 (s, 9H, $C(CH_3)_3$), 0.036 (d, 6H, $Si(CH_3)_2$); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 63.78 (CH_2OSi), 52.44 (CH_2O), 44.45 (CHO), 25.90 ($(CH_3)_3$), 18.38 (CSi), -5.28, -5.32 (CH_3SiCH_3).

1-Phenylsulfonylindole (127).

126 **127**

To a solution of indole **126** (4.8 g, 40 mmol) in dry THF (40 ml) under argon at -78 °C was added dropwise *via* a syringe over 10 min *n*-butyllithium (1.6 M in hexane, 28 ml). After 30 minutes, the cooling bath was removed, and the solution was stirred for 1 h while warming to 0 °C. The resulting indole anion precipitated as a very fine white solid in a cloudy colorless solution. After the suspension was recooled to -78 °C, benzenesulfonyl chloride (5.6 ml, 44 mmol) was added neat via a syringe over 20 minutes. The resulting colorless mixture was allowed to warm slowly to room temperature overnight. Then saturated NH_4Cl solution (60 ml) was added. The mixture was extracted with ethyl acetate (2 x 50 ml). The combined extracts were washed with saturated sodium bicarbonate (60 ml), water (2 x 50 ml), dried over anhydrous sodium sulfate, and evaporated to give a light amber oil which was crystallized when triturated with 2:1 hexane-ether (30 ml). After standing in cold (-20 °C) for several hours, the product was collected by filtration, washed with hexane, and dried in vacuo to provide pure 1-phenylsulfonylindole **127** as white crystals (9.6 g, 90.6%): m.p. 73.0 - 73.5 °C (lit. m.p. 76 - 76.5 °C); ^1H NMR (270 MHz, $CDCl_3$) δ 7.16 - 7.98 (m, 9H, aromatic H), 7.63 (d, ^3J = 3.7, 1H, H-2-indole), 6.71 (d, ^3J = 3.7, 1H, H-3-indole); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 138.3, 134.9, 133.8, 130.8, 129.3, 126.8, 126.3, 124.7, 123.4, 121.5, 113.6, 109.3; MS (FAB, NBA) 258 ($[M+H]^+$, 85.5).

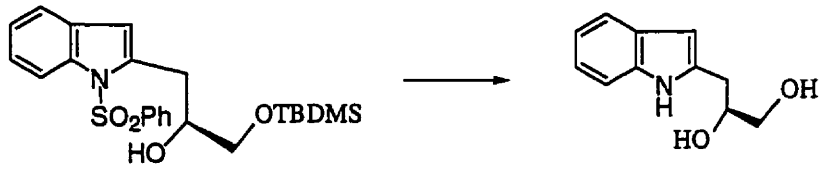
(S)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-isopropanol (128).



125 **127** **128**

To a solution of 1-phenylsulfonyl-indole **127** (6.2 g, 24 mmol) in dry THF (60 ml) was added dropwise *via* a syringe 15 ml of *n*-butyllithium (1.6 M in hexane, 24 mmol) over 10 minutes under argon at -78 ^{0}C. The mixture was stirred for 1.5 h below -70 ^{0}C, then allowed to warm slowly to 5 ^{0}C over 1 h. The solution was cooled to -78 ^{0}C again, and a solution of (S)-glycidyl *tert*-butyldimethylsilyl ether **125** (4.5 g, 24 mmol) in dry THF (10 ml) was added *via* a syringe. The mixture was allowed to warm slowly to room temperature overnight, then poured into saturated NH_4Cl solution (80 ml). The mixture was extracted with ethyl acetate (3 x 40 ml). The combined extracts were washed with H_2O (2 x 100 ml), saturated sodium bicarbonate solution (2 x 100 ml) and brine (2 x 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford a deep red oil. This oil was purified by silica gel chromatography (ethyl acetate/hexane = 1/1) to provide (S)-1-*tert* butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-isopropanol **128** as a pale light yellow solid (5.2 g, 48.4%): m.p. 78 - 79.5 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 - 8.16 (m, 9H, aromatic H), 6.57 (s, 1H, H-3-indole), 4.14 (m, 1H, CHO), 3.74, 3.58 (m, 2H, CH_2OSi), 3.23, 3.09 (m, 2H, CH_2C), 2.57 (d, 1H, ^3J = 4.5 Hz, OH), 0.93 (s, 9H, $(CH_3)_3$), 0.10 (d, 6H, CH_3SiCH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 138.76, 138.13, 137.11, 133.49, 129.64, 129.04, 126.01, 124.01, 123.55, 120.20, 114.75, 111.06 (C_6H_5, C_8H_5), 70.69 (CHOH), 66.32 (CH_2OSi), 32.84 (CH_2C), 25.72 ($(CH_3)_3$), 18.12 (CSi), -5.50, -5.54 (CH_3SiCH_3).

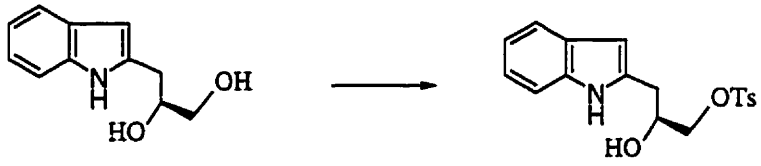
(S)-3-indol-2-yl-propane-1,2-diol (129).



128 **129**

A solution of (S)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-isopropanol **128** (4.5 g, 10.1 mmol) was dissolved in 50 ml of methanol/water (3:1) containing 2.8 g of KOH. The solution was refluxed for 5 h and extracted with ethyl acetate (2 x 50 ml). The combined extracts were washed with H_2O (2 x 100 ml) and brine (2 x 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford pure (S)-3-indol-2-yl-propane-1,2-diol **129** (1.68 g, 86.9%) as a pale solid: m.p. 58.5 - 60 ^{0}C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.58 (s, 1H, NH), 7.53 - 7.00 (m, 4H, C_6H_4), 6.21 (s, 1H, H-3-indole), 3.88 (m, 1H, CHO), 3.56 - 3.40 (m, 2H, CH_2O), 3.2 (s, broad, 1H, OH), 2.79 (m, 2H, CH_2C), 2.00 (s, broad, 1H, OH); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 136.24, 135.71, 128.46, 121.45, 119.96, 119.78, 110.75 (C_6H_4NC), 100.93 (C-3-indole), 71.80 (CHO), 66.06 (CH_2C), 31.87 (CH_2OH).

(S)-3-Indol-2-yl-2-hydroxylpropyl *p*-toluenesulfonate (130)

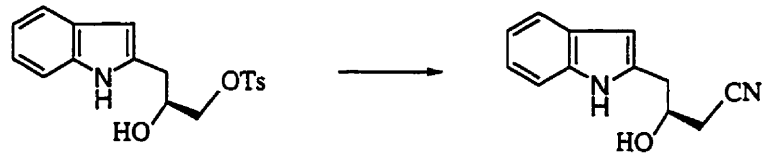


129 **130**

To a solution of (S)-3-indol-2-yl-propane-1,2-diol **129** (1.5 g, 7.9 mmol) in dry pyridine (12 ml) was added *p*-toluenesulfonyl chloride (1.6 g, 8.1 mmol) at 0 ^{0}C. After stirred for 5 h at 0 ^{0}C, the solution was poured into 20 ml of cooled hydrochloric acid (6 N) and extracted with ether (3 x 20 ml). The combined extracts were washed with

hydrochloric acid (6 N, 2 x 20 ml), brine (2 x 20 ml), dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was purified by flash chromatography to give a white solid (S)-3-indol-2-yl-2-hydroxylpropyl *p*-toluenesulfonate **130** (2.45 g, 90%): m.p. 96 - 97 ^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, broad, 1H, NH), 7.78 - 7.06 (m, 8H, aromatic H), 6.21 (s, 1H, H-3-indole), 4.16 (m, 1H, CHO), 4.00 (m, 2H, CH_2O), 2.89 (m, 2H, CH_2C), 2.41 (s, 3H, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 145.15, 136.09, 133.99, 132.77, 129.87, 127.78, 121.36, 119.75, 119.55, 110.56, 101.28, 72.49, 68.98, 31.27, 21.49.

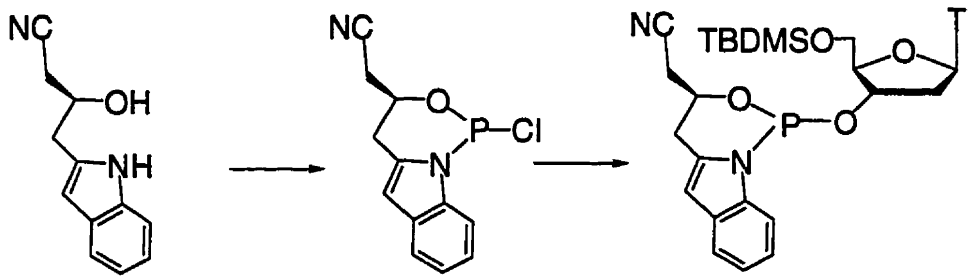
(S)-3-hydroxy-4-(2-indolyl)butyronitrile [28-(S)].



130 **28**-(S)

A solution of (S)-3-indol-2-yl-2-hydroxylpropyl *p*-toluenesulfonate **130** (1.62 g, 4.7 mmol) in DMF (30 ml) containing sodium cyanide (0.5 g, 10.2 mmol) was stirred for 4 h at 100 ^{0}C, then cooled down to room temperature, poured into 80 ml ice-water, and extracted with ethyl acetate (3 x 30 ml). The combined organic solution was washed with saturated sodium bicarbonate (2 x 30 ml), brine (2 x 30 ml), dried over anhydrous sodium sulfate, and evaporated to yield a deep red oil. This oil was purified by flash chromatography (hexanes : ethyl acetate 2:3) to give (S)-3-hydroxyl-4-(2-indolyl)butyronitrile **28-(S)** (0.6 g. 64%) as a light yellow solid: m.p. 78 - 79 ^{0}C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.42 (s, broad, 1H, NH), 7.0-7.5 (m, 4H, C_6H_4), 6.30(d, 1H, CHCN), 4.20 (m, 1H, CHO), 3.01 (m, 2H, CH_2), 2.47, 2.49 (m, 2H, CH_2CN); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 136.56, 133.58, 1218.55, 121.94, 120.16, 120.06, 110.74, 102.12 (C_8H_5N), 117.12 (CN), 67.55 (CH_2C), 35.10 (CHO), 25.28 (CH_2CN); MS (EI) m/e 200 (M, 59.6), 130 (M-$CNCH_2CH_2O$, 100).

Indole Oxazaphosphorine (132a).



28-(S) **131** **132a**

To a solution of PCl_3 (54 μl, 0.62 mmol) in dry THF (2 ml) was added a solution of (S)-3-hydroxyl-4-(2-indolyl)butyronitrile **28**-(S) (124 mg, 0.62 mmol) in THF (1 ml) containing triethylamine (287 μl, 3.3 eq) at 0 °C. After stirred for 30 minutes at 0 °C, the ^{31}P NMR showed that the PCl_3 peak at 220 ppm disappeared and one new peak at 142.86 ppm appeared. To the resulting mixture solution was added a solution of 5'-O-TBDMS-thymidine (220 mg, 0.62 mmol) in THF (2 ml) at 0 °C and stirred for 10 minutes. Then triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 x 10 ml). The filtrate was concentrated and purified by silica gel chromatography (CH_2Cl_2 : CH_3CN = 6 : 1) to afford two diastereomers of indole oxazaphosphorine **132a** as a white solid (145 mg, 40 % yield):

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 120.80, 120.98 (8 : 1 - 12 : 1);

MS(FAB, NBA) m/e 585 ($M+H^+$, 19.7);

The following NMR spectra were assigned for the major one.

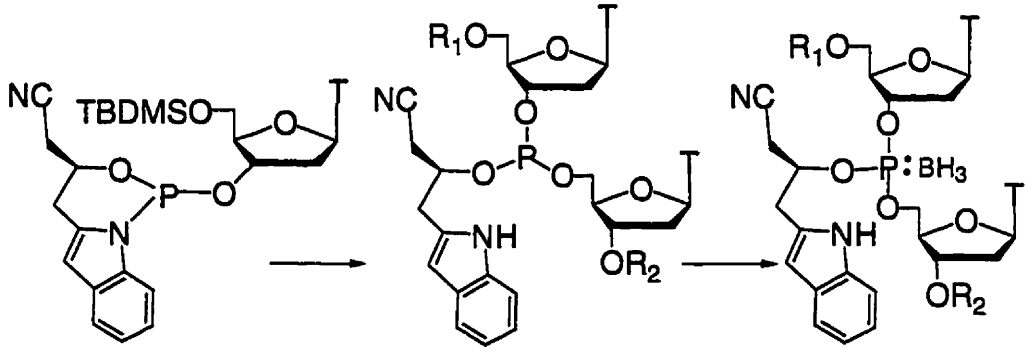
^1H NMR (500 MHz, $CDCl_3$) δ 8.35 (b, 1H, NH), 7.40 (s, 1H, H-6), 7.56- 7.16 (m, 4H, C_6H_4), 6.41 (s, 1H, H-3-indole), 6.32 (dd, 1H, J = 8.0 Hz, J = 5.0 Hz, H-1'), 4.79 (m, 1H, H-3'), 4.47 - 4.45 (m, 1H, CHOP), 3.92 (m, 1H, H-4'), 3.76 - 3.64 (AB, 2H, H-5', H-5''), 3.41 - 3.21 (m, 2H, CH_2C), 2.90 (m, 2H, CH_2CN), 2.41 - 2.37 (m, 1H, H-2'), 2.02 - 1.98 (m, 1H, H-2''), 1.87 (s, 3H, CH_3C-5), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.053, 0.048 (d, 6H, $Si(CH_3)_2$);

COSY (500 MHz, $CDCl_3$) (H-6, CH_3C-5), (H-1', H-2'), (H-1', H-2''), (H-3', H-2'), (H-3', H-2''), (H-3', H-4'), (CHOP, CH_2C), (CHOP, CH_2CN), (H-4', H-5'), (H-4', H-5''), (H-5', H-5''), (H-5'', CH_2C), (H-2', H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.53 (C-4), 150.29 (C-2), 137.95, 137.82, 135.04 (C-6), 133.51, 129.57, 129.54, 122.68, 121.84, 121.76, 120.66, 116.04 (CN), 111.10 (C-5), 110.38, 110.30, 104.32 (C-3-indole), 86.29 (d, J = 3.7 Hz, C-4'), 84.70 (C-1'), 75.32 (d, J = 12.8 Hz, C-3'), 70.54 (d, J = 8.2 Hz, CHOP), 63.05 (C-5'), 39.75 (d, J = 3.7 Hz, C-2'), 30.74 (d, J = 5.5 Hz, $\underline{C}H_2C$), 25.86 ($SiC\underline{Me}_3$), 18.25 ($Si\underline{C}Me_3$), 12.55($\underline{C}H_3C$-5), -5.42 ($SiCH_3$), -5.53 ($SiCH_3$).

HMQC (500 MHz, $CDCl_3$) (H-6, C-6), (H-3-indole, C-3-indole), (H-4', C-4'), (H-1', C-1'), (H-3', C-3'), (C**H**OP, $\underline{C}$HOP), (H-5', H-5'', C-5'), (H-2', H-2'', C-2'), (C$\underline{H_2}$C, $\underline{C}H_2C$), (C$\underline{H_2}$CN, $\underline{C}H_2CN$), (CH_3C-5, CH_3C-5).

Dithymidyl Boranophosphate Triester (134a).



132a **133a** **134a**

R_1 = TBDMS, R_2 = TBDPS

To a solution of indole oxazaphosphorine **132a** (189 mg, 0.33 mmol) in dry THF (2 ml) was added 3'-O-TBDPS-thymidine (53 mg, 0.11 mmol) and DBU (33 µl, 2 eq) at

RT. The reaction mixture was stirred at RT for 10 min and passed through a short silica gel column to filter off DBU. The column was eluted with dry CH_2Cl_2/CH_3CN (1:1). The solvent was evaporated to afford a yellow foam **133a**. Without further purification, this crude phosphite triester was redissolved in dry THF (5 ml), and Me_2S-BH_3 in 2.0 M THF (220 µl, 4 eq) was added, and the mixture was stirred for 3 - 4 min. A small portion of the reaction mixture was taken for ^{31}P NMR which showed complete disappearance of phosphite resonances and after a large number of accumulations, the appearance of a broad peak at ~ 117 ppm for boranophosphate **134a**. The solvent was removed from the reaction mixture at room temperature under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2 : Acetone = 5 : 1) to afforde a light yellow of protected boranophosphate **134a** (83 mg, 70% yield):

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.20 ppm;

^{11}B NMR (96.2 MHz, $CDCl_3$) δ -43.92 ppm;

ES(+) MS (MeOH) m/e 1079 ($[M+H]^+$, 36.05), 1078 (46.01), 1077 (14.23), 1080 (32.03);

ES(-) MS (MeOH) m/e 1077 ($[M-H]^-$, 20.64), 1078 (100), 1079 (77.34), 1080 (67.66), 1081 (37.56);

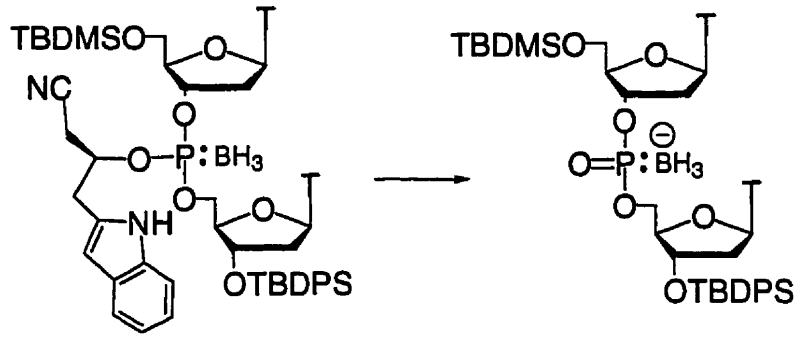
^1H NMR (500 MHz, $CDCl_3$) δ 9.20 (b, 2H, NH), 8.65 (s, 1H, NH), 7.40 (s, 1H, ^5H-6), 6.92 (s, 1H, ^3H-6), 7.64 - 6.95 (m, 14H, aromatic protons), 6.30 (s, 1H, H-3-indole), 6.22 (m, 2H, ^5H-1', ^3H-1'), 4.83 (m, 1H, ^5H-3'), 4.75 (m, 1H, CHOP), 4.24 (m, 2H, ^3H-3', ^3H-4'), 3.93 (m, 1H, ^5H-4'), 3.75 (m, 2H, ^3H-5', ^3H-5''), 3.66 - 3.58 (AB, 2H, ^5H-5', ^5H-5''), 3.24 - 3.10 (AB, 2H, CH_2C), 2.64 -2.58 (m, 2H, CH_2CN), 2.28 - 2.23 (m, 2H, ^5H-2', ^5H-2''), 2.02 - 1.94 (m, 1H, ^3H-2'), 1.93 - 1.79 (m, 1H, ^3H-2''), 1.88 (s, 3H, $^5CH_3C$-5), 1.82 (s, 3H, $^3CH_3C$-5), 1.05 (s, 9H, $SiCMe_3$), 0.88 (s, 9H, $SiCMe_3$), 0.065, 0.058 (d, 6H, $SiMe_2$), 0.4 (b, 3H, BH_3);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (CHOP, CH_2C), (CHOP, CH_2CN), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H-5''), (^{5}H-4', ^{5}H-5'), (^{5}H-4', ^{5}H-5''), (^{5}H-4', CH_2C), (^{5}H-4', CH_2CN), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.66 (^{5}C-4, ^{3}C-4), 150.44 (^{5}C-2), 150.24 (^{3}C-2), 136.26 (^{3}C-6), 136.00(^{5}C-6), 135.70, 135.67, 134.68, 132.81, 132.67, 131.40, 130.28, 130.22, 128.06, 128.00, 127.88, 115.68 (CN), 111.44, 111.37, 102.55 (C-3-indole), 86.19 (^{5}C-1'), 85.70 (d, J = 4.6 Hz, ^{5}C-4'), 84.44 (^{3}C-1'), 84.85 (d, J = 6.3 Hz, ^{3}C-4'), 79.75 (^{5}C-3'), 72.90 (^{3}C-3'), 72.80 (CHOP), 66.28 (^{3}C-5'), 63.14 (^{5}C-5'), 39.44 (^{5}C-2'), 39.14 (^{3}C-2'), 33.83 ($\underline{C}H_2$C), 26.78 (SiC$\underline{Me}_3$), 25.85 (SiC$\underline{Me}_3$), 23.82 ($\underline{C}H_2$CN), 18.96 (Si$\underline{C}Me_3$), 18.23 (Si$\underline{C}Me_3$), 12.50 ($^3\underline{C}H_3$C-5), 12.34 ($^5\underline{C}H_3$C-5), -5.46 (Si$\underline{Me}_2$), -5.55(Si$\underline{Me}_2$);

HMQC (500 MHz, $CDCl_3$) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (H-3-indole, C-3-indole), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (C$\underline{H}$OP, $\underline{C}$HOP), (^{3}H-3', ^{3}C-3'), (^{5}H-3', ^{5}C-3'), (^{3}H-4', ^{3}C-4'), (^{5}H-4', ^{5}C-4'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (C$\underline{H}_2$C, $\underline{C}H_2$C), (SiC$\underline{Me}_3$, SiC$\underline{Me}_3$), (C$\underline{H}_2$CN, $\underline{C}H_2$CN), (^{5}C$\underline{H}_3$C-5, $^5\underline{C}H_3$C-5), (^{3}C$\underline{H}_3$C-5, $^3\underline{C}H_3$C-5).

Dithymidyl boranophosphate, ammonium salt (133).



134a **135**

The dimer **134a** (10 mg, 9 μmol) was taken in conc. NH_4OH (2 ml) in a sealed tube. The mixture was shaken overnight at RT. The tube was cooled in ice and opened to atmosphere. After allowing the ammonia to escape, the solution was lyophilized to give a white solid. After chromatography with ethyl acetate/methanol (10/1 - 3/1), a pure compound **135** was obtained (5.9 mg, 73% yield):

^{31}P NMR (121 MHz, MeOH) δ 96.53 ppm;

^{11}B NMR (96.2 MHz, MeOH) δ -40.81 ppm;

ES(-) MS (TEA/H_2O) m/e 894.2 ($[M-H]^-$, 23.69), 895.2 (100), 896.2 (59.90), 897.2 (35.64), 898.2 (14.18);

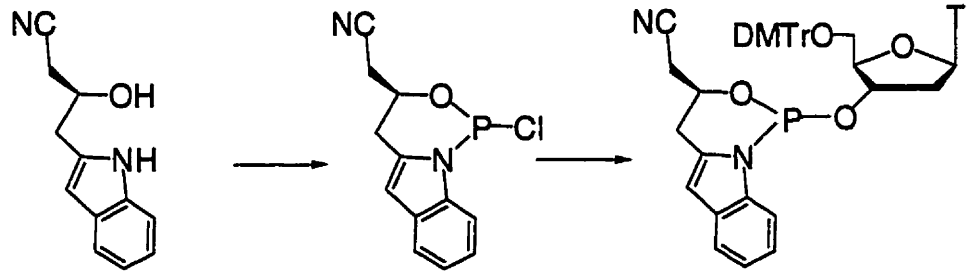
^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, 1H, J = 1.2 Hz, ^{5}H-6), 7.61 (d, 1H, J = 1,0 Hz, ^{3}H-6), 7.67 - 7.63 (m, 2H, aromatic protons), 7.45 - 7.37 (m, 8H, aromatic protons), 6.49 (dd, 1H, J = 5.6, 5.6 Hz, ^{5}H-1'), 6.20 (dd, 1H, J = 5.4, 5.4 Hz, ^{3}H-1'), 4.83 (m, 1H, ^{3}H-3'), 4.59 (m, 1H, ^{5}H-3'), 4.12(m 1H, ^{3}H-4'), 4.03 (m, 1H, ^{5}H-4'), 3.90 - 3.79 (m, 1H, ^{3}H-5'), 3.78 - 3.70 (m, 2H, ^{5}H-5', ^{5}H-5''), 3.61 - 3.57 (m 1H, ^{3}H-5''), 2.26 - 2.21 (m, 2H, ^{3}H-2',), 2.20 - 2.14 (m, 1H, ^{5}H-2') 2.13 - 2.00 (m, 1H, ^{5}H-2''), 1.99 - 1.93 (m, 1H, ^{3}H-2''), 1.91 (d, 3H, J = 1.0 Hz, $^5CH_3$C-5), 1.87 (d, 3H, J = 1.2 Hz, $^3CH_3$C-5), 1.08 (s, 9H, $SiCMe_3$), 0.91 (s, 9H, $SiCMe_3$), 0.38 (b, 3H, BH_3), 0.11, 0.10 (d, 6H, $SiMe_2$);

COSY (500 MHz, $CDCl_3$) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (^{3}H-4', ^{3}H-5'), (^{3}H-4', ^{3}H-5''), (^{5}H-4', ^{5}H-5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, CD_3OD) 138.16 (^{5}C-6), 137.38 (^{3}C-6), 136.92, 131.24, 131.17, 129.14, 129.05 (aromatic carbons), 88.46 (^{3}C-4'), 88.20 (^{5}C-4'), 86.43 (^{3}C-1'), 86.40 (^{5}C-1'), 76.50 (^{3}C-3'), 76.4 (^{5}C-3'), 64.88 (^{5}C-5'), 63.00 (^{3}C-5'), 41.58 (^{5}C-2'), 41.04 (^{3}C-2'), 27.44 (SiC**Me$_3$**), 26.53 (SiC**Me$_3$**), 12.71 (^{3}**C**H$_3$C-5), 12.50 (^{5}**C**H$_3$C-5);

HMQC (500 MHz, CD_3OD) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (aromatic protons, aromatic carbons), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (^{3}C**H$_3$**C-5, ^{3}**C**H$_3$C-5), (^{5}C**H$_3$**C-5, ^{5}**C**H$_3$C-5), (SiC**Me$_3$**, SiC**Me$_3$**).

Indole Oxazaphosphorine (132b).



28-(S) **131** **132b**

To a solution of PCl_3 (54 µl, 0.62 mmol) in dry THF (2 ml) was added a solution of (S)-3-hydroxy-4-(2-indolyl)butyronitrile **28**-(S) (124 mg, 0.62 mmol) in THF (1 ml)

containing triethylamine (287 µl, 3.3 eq) at 0 ^{0}C. After stirred for 30 minutes at 0 ^{0}C, the ^{31}P NMR showed that the PCl_3 peak at 220 ppm disappeared and one new peak at 142.86 ppm appeared. To the resulting mixture solution was added a solution of 5-O'-DMTr-thymidine (337 mg, 0.62 mmol) in THF (2 ml) at 0 ^{0}C and stirred for 10 minutes. Then triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 x 10 ml). The filtrate was concentrated and purified by silica gel chromatography (CH_2Cl_2 : CH_3CN = 6: 1, then CH_2Cl_2 : CH_3CN = 1 : 1) to afford a white solid of two diastereoisomers of indole oxazaphosphorine **132b** (215 mg, 45 % yield);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 121.66, 122.23 (16 : 1);

LRMS (FAB-NBA/NaCl) m/e 773 ($[M+H]^+$, 1.0), 795 ($[M+Na]^+$, 4.6);

The following NMR spectra were assigned for the major isomer:

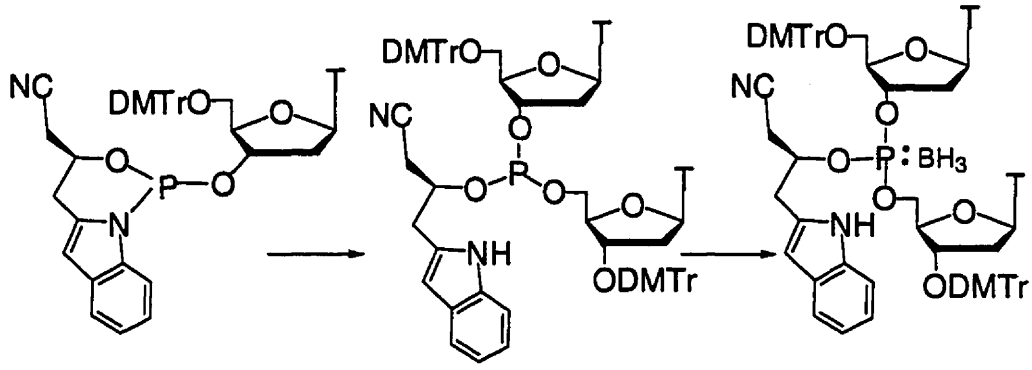
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (b, 1H, NH), 7.53 (s, 1H, H-6), 7.39 - 6.80 (m, 17H, aromatic protons), 6.40 (s, 1H, H-3-indole), 6.38 (dd, 1H, ^{3}J = 8.8, 5.0 Hz, H-1'), 4.94 (m, 1H, H-3'), 4.40 (m, 1H, CHOP), 4.02 (m, 1H, H-4'), 3.78 (d, 6H, 2xCH_3O), 3.49 - 3.27 (AB, 2H, H-5', H-5''), 3.15 - 3.03 (m, 2H, CH_2C), 2.66 (m, 2H, CH_2CN), 2.47, 2.00 (m, 2H, H-2', H-2''), 1.39 (s, 3H, CH_3C-5);

COSY (500 MHz, $CDCl_3$) (H-6, CH_3C-5), (H-1', H-2'), (H-1', H-2''), (H-3', H-2'), (H-3', H-2''), (H-3', H-4'), (CHOP, CH_2C), (CHOP, CH_2CN), (H-4', H-5'), (H-4', H-5''), (H-5', H-5''), (H-2', H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.42 (C-4), 158.77, 150.20 (C-2), 144.06, 137.89, 137.76, 135.38 (C-6), 135.11, 135.06, 133.71, 130.11, 129.60, 129.58, 128.20, 128.15, 128.01, 127.96, 127.24, 122.73, 121.79, 120.60, 115.87 (CN), 113.28, 113.27, 113.23, 111.45 (C-5), 110.45, 110.37, 104.34 (C-3-indole), 87.05 (C-O-C-5'), 85.32 (d, J = 2.8 Hz, C-4'), 84.61 (C-1'), 75.16 (d, J = 10.1 Hz, C-3'), 70.28 (d, J = 7.3 Hz, CHOP), 62.90 (C-5'), 55.27 (CH_3O), 39.57 (d, J = 2.7 Hz, C-2'), 30.88 (d, J = 5.5 Hz, CH_2C), 25.29 (d, J = 3.7 Hz, CH_2CN), 11.69 (CH_3C-5);

HMQC (500 MHz, $CDCl_3$) (H-6, C-6), (H-3-indole, C-3-indole), (H-1', C-1'), (H-4', C-4'), (H-3', C-3'), (**CH**OP, **C**HOP), (H-5', H-5'', C-5'), (**CH$_3$**O, **C**H$_3$O), (H-2', H-2'', C-2'), (**CH$_2$**C, **C**H$_2$C), (**CH$_2$**CN, **C**H$_2$CN), (**CH$_3$**C-5, **C**H$_3$C-5).

Dithymidyl Boranophosphate triester (134b).



132b **133b** **134b**

To a solution of indole oxazaphosphorine **132b** (169 mg, 0.22 mmol) in dry THF (2 ml) was added 3'-O-DMTr-thymidine (117 mg, 0.22 mmol) and DBU (66 µl, 2 eq) at RT. Within 10 min, the TLC showed that the reaction has completed. Then, the mixture was passed through a short silica gel column to filter off DBU, and flashed with dry CH_2Cl_2/CH_3CN (1:1). The solvent was evaporated to afford a yellow foam. ^{31}P NMR showed that two peaks at 140.95 : 140.86 (10 : 1). Without further purification, this crude phosphite triester **133b** was redissolved in dry THF (5 ml), and Me_2S-BH_3 in 2.0 M THF (440 µl, 4 eq) was added, and the mixture was stirred at RT for 3 - 4 min. A small portion of the reaction mixture was taken for ^{31}P NMR which showed complete disappearance of phosphite resonances and, after a large number of accumulations, the appearance of a broad peak at ~ 117 ppm for boranophosphate. After 3 h, the solvent was removed from the reaction mixture at room temperature under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2 : Acetone = 5 : 1) to give the protected boranophosphate **134b** (two diastereoisomers in a ratio of 10 : 1, 210 mg 72% yield;

When using 3 eq. of indole oxazaphosphorine **132b** (254 mg, 0.33 mmol) and 1 eq. of 3'-O-DMTr-thymidine (59 mg, 0.11 mmol), the first step showed one single peak at 140.90 ppm in ^{31}P NMR, and the second step afforded the protected boranophosphate **134b** (one diastereoisomer, 95 mg, 65% yield):

The following NMR spectra were assigned for one isomer,

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 117.06 ppm;

^{11}B NMR (96.2 MHz, $CDCl_3$) δ -43.57 ppm;

LRMS (FAB, NBA): m/e 1331 $[M + H]^+$;

^1H NMR (500 MHz, $CDCl_3$) δ 9.00 (br s, 1H, NH), 8.51 (br s, 1H, NH), 7.45 (s, 1H, ^5H-6), 6.93 (s, 1H, ^3H-6), 7.41 - 6.80 (m, 30H, aromatic protons), 6.27 (s, 1H, H-3-indole), 6.27 - 6.24 (dd, 1H, ^5H-1'), 6.11 (dd, J = 7.6 Hz, J = 5.6 Hz, 1H, ^3H-1'), 5.01 (m, 1H, ^5H-3'), 4.64 (m, 1H, CHOP), 4.16 (m, 1H, ^3H-3'), 3.98 (m, 1H, ^5H-4'), 3.96 (m, 1H, ^3H-4'), 3.751, 3.747, 3.743, 3.740 (4s, 12H, 4xCH_3O), 3.76 - 3.60 (m, 2H, ^5H-5'x2), 3.30 - 3.22 (AB, 2H, ^3H-5'x2), 3.13 - 2.96 (AB, 2H, CH_2C), 2.59 -2.47 (AB, 2H, CH_2CN), 2.39 - 2.35 (m, 1H, ^5H-2'), 2.23 - 2.18 (m, 1H, ^5H-2''), 1.99 - 1.69 (m, 2H, ^3H-2', ^3H-2''), 1.79 (s, 3H, $^3CH_3C$-5), 1.43 (s, 3H, $^5CH_3C$-5), 0.40 (b, 3H, BH_3);

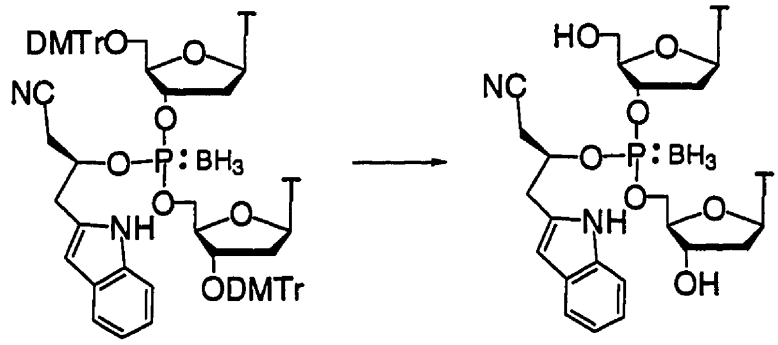
COSY (500 MHz, $CDCl_3$) (^5H-6, $^5CH_3C$-5), (^3H-6, $^3CH_3C$-5), (^5H-1', ^5H-2'), (^5H-1', ^5H-2''), (^3H-1', ^3H-2'), (^3H-1', ^3H-2''), (CHOP, CH_2C), (CHOP, CH_2CN), (^5H-3', ^5H-2''), (^5H-3', ^5H-4'), (^3H-3', ^3H-2'), (^3H-3', ^3H-2''), (^3H-3', ^3H-4'), (^3H-4', ^3H-5'), (^3H-4', ^3H-5''), (^5H-4', ^5H-5'), (^5H-4', ^5H-5''), (^5H-2', ^5H-2''), (^3H-2', ^3H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.55 (^5C-4), 163.51 (^3C-4), 158.79, 158.75, 150.37 (^5C-2), 150.16 (^3C-2), 144.79, 143.98, 136.23, 135.87 (^3C-6), 135.00(^5C-6), 134.94, 130.24, 130.03, 128.11, 128.09, 127.99, 115.64 (CN), 113.49, 113.47, 113.38, 111.76, 111.26, 110.79, 102.50 (C-3-indole), 87.39, 87.27, 86.87 (^3C-1'), 84.59 (d, J = 5.5 Hz, ^5C-4'), 84.36 (^5C-1'), 83.82 (d, J = 5.5 Hz, ^3C-4'), 79.38 (^5C-3'), 73.82 (^3C-

3'), 72.69 (d, J = 2.7 Hz, CHOP), 69.49, 66.73 (d, J = 4.6 Hz, ^{5}C-5'), 63.18 (^{3}C-5'), 55.27 (CH_3O), 38.95 (^{5}C-2'), 38.10 (^{3}C-2'), 33.72 ($\underline{C}H_2C$), 23.62 ($\underline{C}H_2CN$), 12.35 ($^3\underline{C}H_3C$-5),, 11.77 ($^5\underline{C}H_3C$-5);

HMQC (500 MHz, $CDCl_3$) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (H-3-indole, C-3-indole), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (C$\underline{H}$OP, $\underline{C}$HOP), (^{3}H-3', ^{3}C-3'), (^{5}H-3', ^{5}C-3'), (^{3}H-4', ^{3}C-4'), (^{5}H-4', ^{5}C-4'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (C$\underline{H}_3$O, $\underline{C}H_3$O), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (C$\underline{H}_2$C, $\underline{C}H_2$C), (C$\underline{H}_2$CN, $\underline{C}H_2$CN), (^{5}C$\underline{H}_3$C-5, $^5\underline{C}H_3$C-5), (^{3}C$\underline{H}_3$C-5, $^3\underline{C}H_3$C-5).

Deprotected Dithymidyl boranophosphate (137).



134b **137**

A solution of protected boranophosphate **134b** (a mixture of two diastereomers or one diastereomer obtained from above reactions) (36 mg, 27 μmol) in 1.4 ml HOAc/0.6 ml H_2O/1 ml MeOH was stirred until the removal of dimethoxytrityl groups has completed. The solvent was evaporated and then coevaporated with methanol twice. The mixture was chromatographed on a silica gel column (EtOAc/MeOH = 100/0 - 95/5) to give the boranophosphate dimer **137** (11 mg, 56 % yield):

The following NMR spectra were assigned for one isomer:

^{31}P NMR (202.3 MHz, CD_3OD) δ 116.56 ppm;

^{11}B NMR (96.2 MHz, CD_3OD) δ -43.87 ppm;

HRMS (FAB, Glycerol) m/e calcd for $C_{32}H_{41}N_6O_{11}PB$ $[M + H]^+$ 727.2664, found 727.2666;

^1H NMR (500 MHz, CD_3OD) δ 7.57 (d, J = 1 Hz, 1H, ^5H-6), 7.39 (d, J = 1 Hz, 1H, ^3H-6), 7.48 - 6.80 (m, 4H, aromatic protons), 6.34 (s, 1H, H-3-indole), 6.24 (m, 1H, ^5H-1'), 6.07 (m, 1H, ^3H-1'), 4.98 (m, 1H, CHOP), 4.82 (m, 1H, ^3H-3'), 4.32 (m, 1H, ^5H-3'), 4.17 (m, 1H, ^5H-5'), 4.08 (m, 1H, ^5H-5''), 3.95 (m, 1H, ^5H-4'), 3.86 (m, 1H, ^3H-4'), 3.77(m, 1H, ^3H-5'), 3.53(m, 1H, ^3H-5''), 3.28 - 3.19 (AB, 2H, CH_2C), 3.07 - 2.90 (AB, 2H, CH_2CN), 2.24 - 2.15 (m, 2H, ^5H-2'', ^5H-2'), 2.10 - 2.06 (m, 1H, ^3H-2''), 1.91 - 1.82 (m, 1H, ^3H-2'), 1.88 (d, J = 1 Hz, 3H, $^5CH_3C$-5), 1.86 (d, J = 1 Hz, 3H, $^3CH_3C$-5), 0.41 (b, 3H, BH_3);

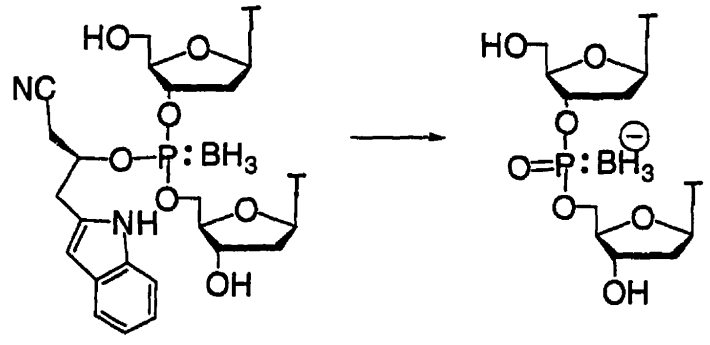
COSY (500 MHz, CD_3OD) (^5H-6, $^5CH_3C$-5), (^3H-6, $^3CH_3C$-5), (^5H-1', ^5H-2'), (^5H-1', ^5H-2''), (^3H-1', ^3H-2'), (^3H-1', ^3H-2''), (CHOP, CH_2C), (CHOP, CH_2CN), (^3H-3', ^3H-2''), (^3H-3', ^3H-4'), (^5H-3', ^5H-2'), (^5H-3', ^5H-2''), (^5H-3', ^5H-4'), (^5H-5', ^5H-5''), (^5H-5', ^5H -4'), (^5H-5'', ^5H -4'), (^3H-4', ^3H-5''), (^5H-2', ^5H-2''), (^3H-2', ^3H-2'');

^{13}C NMR (125.7 MHz, CD_3OD) δ 166.29 (C-4), 152.25 (^5C-2), 152.22 (^3C-2), 137.73 (^5C-6), 137.54 (^3C-6), 122.29, 120.88, 120.36, 117.61 (CN), 112.07, 111.95 (^5C-5), 111.80 (^3C-5), 102.55 (C=<u>C</u>H-Ph), 87.05 (d, J = 3.6 Hz, ^3C-4'), 86.30 (^5C-1'), 85.89 (^3C-1'), 85.81 (d, J = 9.1 Hz, ^5C-4'), 79.94 (d, J = 2.8 Hz, ^3C-3'), 74.62 (d, J = 2.8 Hz, CHOP), 71.73 (^5C-3'), 67.33 (d, J = 4.6 Hz, ^5C-5'), 62.44 (^3C-5'), 40.32 (^5C-2'), 39.49 (^3C-2'),, 34.90 (d, J = 2.8 Hz, <u>C</u>H_2C) , 25.14 (<u>C</u>H_2CN), 12.67 (^{5}<u>C</u>H_3C-5), 12.48 (^{3}<u>C</u>H_3C-5);

HMQC (500 MHz, CD_3OD) (^5H-6, ^5C-6), (^3H-6, ^3C-6), (C=C<u>H</u>-Ph, C=C<u>H</u>-Ph), (^5H-1', ^5C-1'), (^3H-1', ^3C-1'), (C<u>H</u>OP, <u>C</u>HOP), (^3H-3', ^3C-3'), (^5H-3', ^5C-3'), (^3H-4', ^3C-4'), (^5H-4', ^5C-4'), (^5H-5', ^5H-5'', ^5C-5'), (^3H-5', ^3H-5'', ^3C-5'), (C<u>H</u>$_3$O, <u>C</u>H_3O), (^5H-2',

^{5}H-2'', ^{5}C-2'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (C$\underline{H}_2$C, $\underline{C}H_2$C), (C$\underline{H}_2$CN, $\underline{C}H_2$CN), (^{5}C$\underline{H}_3$C-5, $^5\underline{C}H_3$C-5), (^{3}C$\underline{H}_3$C-5, $^3\underline{C}H_3$C-5).

Dithymidyl boranophosphate, ammonium salt (Sp-115/Rp-116).



137 Sp-**115**/Rp-**116**

The dimer **137** (a mixture of two diastereomers or one diastereomer obtained from above reactions) (10 mg, 14 μmol) was taken in conc. NH_4OH (2 ml) in a sealed tube. The mixture was shaken at RT for 1 h. The tube was cooled in ice and opened to atmosphere. After allowing the ammonia to escape, the solution is lyophilized to give a white solid or the solvent was removed in high vacuum rotary evaporator. The crude product was purified with silica gel column (EtOAc/MeOH = 1/0 ~ 10/1) or washed with ethyl acetate several times to afford the dithymidine boranophosphate Sp-**115**/Rp-**116** (6.1 mg, 80% yield):

The following NMR spectra were assigned for Sp-**115**:

^{31}P NMR (202.3 MHz, D_2O) δ 93.51 ppm;

^{11}B NMR (96.2 MHz, D_2O) δ - 41.64 ppm;

ES(-) MS (TEA/H_2O) m/e 542.2 ([M-H]$^-$, 22.18), 543.2 (100), 544.1 (72.80), 545.2 (33.64), 546.2 (11.57);

^{1}H NMR (500 MHz, D_2O) δ 7.51 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.48 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.17 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{5}H-1'), 6.07 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{3}H-1'), 4.68 - 4.64 (m, 1H, ^{3}H-3'), 4.43 - 4.41 (m, 1H, ^{5}H-3'), 4.00 - 3.97 (m, 1H, ^{3}H-

4', ^{5}H-4'), 3.94 - 3.92 (m, 2H, ^{5}H-5', ^{5}H-5''), 3.69 -3.59 (AB, 2H, ^{3}H-5', ^{3}H-5''), 2.36 - 2.31 (m, 1H, ^{3}H-2'), 2.22 (m, 2H, ^{5}H-2', ^{5}H-2''), 2.20 - 2.16 (m, 1H, ^{3}H-2''), 1.75 (d, J = 1 Hz, 3H, $^5CH_3$C-5), 1.71 (d, J = 1 Hz, 3H, $^3CH_3$C-5), 0.20 (b, 3H, BH_3);

COSY (500 MHz, D_2O) (^{5}H-6, $^5CH_3$C-5), (^{3}H-6, $^3CH_3$C-5), (^{5}H-1', ^{5}H-2'), (^{5}H-1', ^{5}H-2''), (^{3}H-1', ^{3}H-2'), (^{3}H-1', ^{3}H-2''), (^{3}H-3', ^{3}H-2'), (^{3}H-3', ^{3}H-2''), (^{3}H-3', ^{3}H-4'), (^{5}H-3', ^{5}H-2'), (^{5}H-3', ^{5}H-2''), (^{5}H-3', ^{5}H-4'), (^{3}H-4', ^{3}H-5''), (^{3}H-4', ^{3}H -5''), (^{5}H-4', ^{5}H -5'), (^{5}H-4', ^{5}H-5''), (^{5}H-2', ^{5}H-2''), (^{3}H-2', ^{3}H-2'');

^{13}C NMR (125.7 MHz, D_2O) δ 166.18 (^{5}C-4), 166.05 (^{3}C-4), 151.43 (^{5}C-2), 151.23(^{3}C-2), 137.07 (^{5}C-6, ^{3}C-6), 111.26 (^{5}C-5), 111.19 (^{3}C-5) , 85.56 (d, J = 4.6 Hz, ^{3}C-4'), 85.03 (d, J = 6.4 Hz, ^{5}C-4'), 84.88 (^{3}C-1'), 84.45 (^{5}C-1'), 72.61 (d, J = 3.0 Hz, ^{3}C-3'), 70.42 (^{5}C-3'), 61.37 (d, J = 4.6 Hz, ^{5}C-5'), 60.55 (^{3}C-5'), 38.41 (^{5}C-2'), 37.60 (^{3}C-2'), 11.50 (^{5}**C**H_3C-5), 11.29 (^{3}**C**H_3C-5);

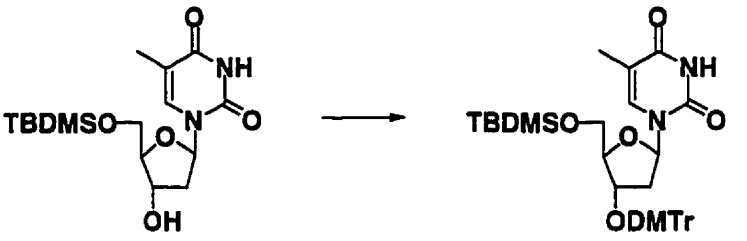
HMQC (500 MHz, D_2O) (^{5}H-6, ^{5}C-6), (^{3}H-6, ^{3}C-6), (^{5}H-1', ^{5}C-1'), (^{3}H-1', ^{3}C-1'), (^{5}H-4', ^{5}C-4'), (^{3}H-4', ^{3}C-4'), (^{5}H-3', ^{5}C-3'), (^{3}H-3', ^{3}C-3'), (^{5}H-5', ^{5}H-5'', ^{5}C-5'), (^{3}H-5', ^{3}H-5'', ^{3}C-5'), (^{5}H-2', ^{5}H-2'', ^{5}C-2'), (^{3}H-2', ^{3}H-2'', ^{3}C-2'), (^{5}C**H**$_3$C-5, ^{5}**C**H_3C-5); (^{3}C**H**$_3$C-5, ^{3}**C**H_3C-5).

The Synthesis of 3'-O-(4,4'-dimethoxyltrityl)thymidine:

5'-O-(*tert*-butyldimethylsilyl) thymidine

See Chapter 2: Section 2.5.3.

3'-O-(4,4'-dimethoxytrityl)-5'-O-(*tert*-butyldimethylsilyl)-thymidine

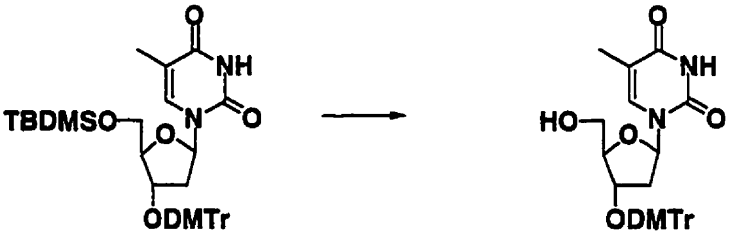


Use the same procedure as described for the preparation of 5'-O-(4,4'-dimethoxytrityl) thymidine (See Chapter 2: Section 2.5.3).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (b, 1H, NH), 7.43 - 6.79 (m, 13H, Ph), 7.22 (d, J = 1.2 Hz, 1H, H-6), 6.39 (dd, J = 5.4 Hz, J = 5.4 Hz, 1H, H-1'), 4.24 (m, 1H, H-3'), 4.00 (m, 1H, H-4'), 3.77 (s, 6H, OCH_3 x 2), 3.72 - 3.25 (AB, 2H, H-5', H-5''), 1.83 (d, J = 1.2 Hz, 3H, CH_3C-5), 1.72 -1.67 (m, 1H, H-2'), 1.57 - 1.51 (m, 1H, H-2''), 0.78 (s, 9H, CMe_3), -0.072 (s, 3H, SiMe), -0.124 (s, 3H, SiMe);

MS (FAB, NBA) m/e 659 ($[M+ H]^+$, 9.9).

3'-O-(4,4'-dimethoxytrityl) thymidine



To a solution of 3'-O-(4,4'-dimethoxytrityl)-5'-O-(*tert*-butyldimethylsilyl)-thymidine (436 mg, 0.66 mmol) in 10 ml of THF was added TBAF (2 ml, 1.0 M) solution in THF at rt. The mixture was stirred at rt overnight. The reaction was quenched with water, then the mixture was extracted with EtOAc, washed with water and dried over $MgSO_4$. The flash

chromatography (hexanes/ethyl acetate/triethylamine = 2/1/1 - 0/2/1) of crude product gave a light yellow powder of 3'-O-(4,4'-dimethoxytrityl) thymidine (357 mg, 99%):

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (b, 1H, NH), 7.22 (d, J = 1.2 Hz, 1H, H-6), 7.43 - 6.80 (m, 13H, Ph), 6.11 (dd, 1H, J = 5.9 Hz, J = 5.9 Hz, H-1'), 4.34 (m, 1H, H-3'), 3.95 (m, 1H, H-4'), 3.77 (s, 6H, OCH_3 x 2), 3.65 - 3.29 (AB, 2H, H-5', H-5''), 2.43 (b, 1H, OH), 1.94 - 1.88 (m, 1H, H-2'), 1.70 - 1.65 (m, 1H, H-2''), 1.83 (d, J = 1.2 Hz, 3H, CH_3C-5);

COSY (500 MHz, $CDCl_3$): (H-6, CH_3C-5), (H-1', H-2'), (H-1', H-2''), (H-3', H-4'), (H-3', H-2'), (H-3', H-2''), (H-4', H-5'), (H-4', H-5''), (H-5', H-5''), (H-5'', OH), (H-2', H-2'');

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.56 (C-4), 158.69, 158.67, 150.26 (C-2), 145.04, 137.14 (C-6), 136.27, 136.18, 130.21, 130.17, 128.23, 127.96, 127.07, 113.29, 113.26, 110.92 (C-5), 87.48 (C-4'), 87.18 (C-O-C-5'), 86.54 (C-1'), 74.22 (C-3'), 62.57 (C-5'), 55.23 (OCH_3), 38.56 (C-2'), 12.44 (CH_3);

MS (FAB, NBA) m/e 545 ($[M+H]^+$, 0.9).

4.6. References

1. (a) Sood, A.; Shaw, B. R.; Speilvogel, B. F. *J. Am. Chem. Soc.* **1990**, *112*, 9000; (b) Sergueev, D.; Hasan, A.; Ramaswamy, M.; Shaw, B. R. *Nucleosides & Nucleotides* **1997**, *16*, 1533.
2. Wang, J.-C.; Just, G. *Tetrahedron Lett.* **1997**, *38*, 3797.
3. Shaw, B. R.; Madison, J.; Sood, A.; Spielvogel, B. F. *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs* Edited by: S. Agrawal, copyright, **1993**, Humana Press Inc., Totowa, NJ.
4. see Chapter 3 and references therein.
5. (a) Fuji, M.; Ozaki, K.; Sekine, M. and Hata, T. *Tetrahedron* **1987**, *43*, 3395. (b) Mikolajczyk, M. *J. Chem. Soc., Chem. Commun.* **1969**, 1221.

Contribution to Original Knowledge

- Novel xyiose-based chiral auxiliaries, namely 1,2-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **51** and its enantiomer **52**, were synthesized.
- A novel methodology for the stereoselective synthesis of Sp and Rp dithymidine phosphorothioates in a de > 98% was developed by the use of the phosphoramidite method.
- It was discovered that the mechanism of the coupling reaction involves only one inversion in the transformation of the phosphoramidites (**60**, **68**) to the dithymidine phosphorothioates (**65**, **66**).
- The study of the effect of the acidity of the catalyst on the stereoselectivity and the rate of the coupling reaction was studied.
- A method was developed for the synthesis of chiral auxiliary **54**. Such a precursor should be a good chiral auxiliary for the stereoselective synthesis of phosphorothioates.

◊ A method was developed to synthesize the diastereomerically pure Sp-**109** and Rp-**110** dimers for the solid phase synthesis of mixed backbone oligonucleotides.

◊ A chemical synthesis method to the diastereomerically pure Sp-**115** and Rp-**116** dithymidine boranophosphates was developed.

* A novel methodology for the stereoselective synthesis of dinucleotide boranophosphates was developed by the use of indole-oxazaphosphorine as chiral precursors. A diastereomerically pure Sp-**115** dithymidine boranophosphate was synthesized in a de > 98%.

* The boronation reaction with dimethyl sulfide-borane was proved to be a stereoretentive process.

Papers Resulting from the Research

1. Jin, Y.; Biancotto, G.; Just, G. "A Stereoselective Synthesis of Dinucleotide Phosphorothioates, Using Chiral Phosphoramidites as Intermediates." *Tetrahedron Lett.* **1996**, *37*, 973 - 976.
2. Jin, Y.; Just, G. "Stereoselective Synthesis of Dithymidine Phosphorothioates Using Xylose Derivatives as Chiral Auxiliaries." *J. Org. Chem.* **1998**, *63*, 3647 - 3654.
3. Jin, Y.; Just, G. "The Synthesis of the Sp and Rp Diastereomers of Dithymidine Boranophosphates." *Tetrahedron Lett.* **1998**, in press.
4. Jin, Y.; Just, G. "Stereoselective Synthesis of Dinucleotide Boranophosphates, Using Chiral Indole-oxazaphosphorine Intermediates." *Tetrahedron Lett.* **1998**, in press.