TOWARDS THE STEREOSELECTIVE SYNTHESIS OF OLIGONUCLEOTIDE PHOSPHOROTHIOATES AND PHOSPHONATE DERIVATIVES

By

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Doctor of Philosophy

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For My Parents, My Brother

Dedicated to My Loving Wife Ying

What you believe to be the summit is only a step further

- Epikur (342-270 B. C.)

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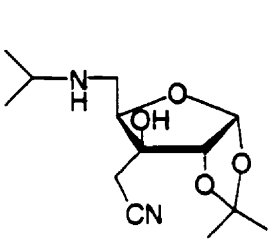
Abstract

Chiral auxiliary 1,2-di-O-isopropylidene-3-C-cyanoethyl-5-deoxy-5-isopropylamino-D-xylofuranose **3-32** was synthesized. Cyclic phosphoramidite **3-34** derived from **3-32** is a useful precursor for the stereoselective synthesis of phosphorothioate. The chiral auxiliary can be easily removed at the end of the synthesis by treatment with concentrated ammonia. The major dithymidine phosphorothioate isomer with a S_P configuration was obtained.

The allyl group was investigated as a protecting group for the internucleotide linkage. The removal of the allyl group in dimer **4-6S** or **4-6O** was achieved by treatment with nucleophiles. Solid phase study indicated this to be a general method for the routine solid phase synthesis of oligonucleotide phosphorothioates and phosphates.



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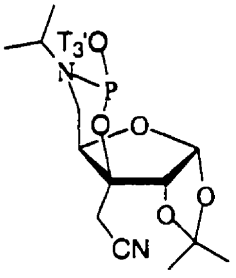

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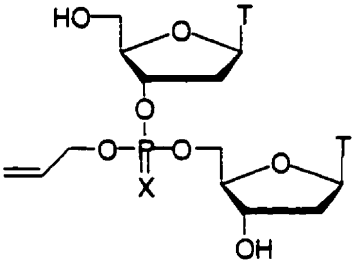
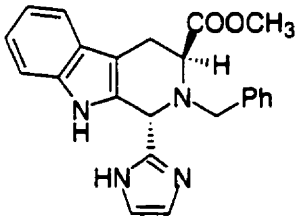


4-6 S X=S, **O** X=O

A new type of chiral auxiliary **5-20** which incorporates indole and imidazole moieties was synthesized. It led to the stereoselective synthesis of a novel indolophosphorothioate **5-37** with 90% de and the formation of an alkylphosphinate analog **5-41** as a single diastereomer.

Chiral indolo-oxazaphosphorine **6-22** was prepared from L-tryptophan. Dithymidine phosphorothioate with ≥95% de was obtained while using **6-22** as a precursor. The removal of the chiral auxiliary was achieved by heating in concentrated ammonia at 55 °C for 16 hours. The indolo-oxazaphosphorines derived from L- or D-tryptophans led to the formations of dithymidine phosphorothioates with R_P or S_P configurations, respectively. The methodology was applied successfully to the solid phase synthesis of phosphorothioate dimers.



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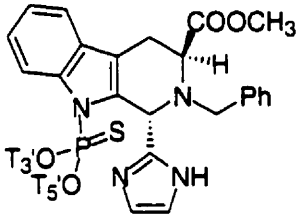

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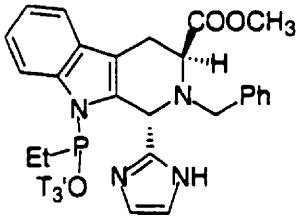

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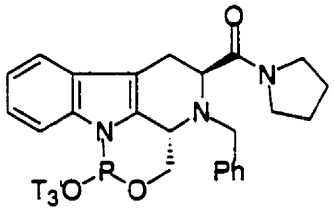
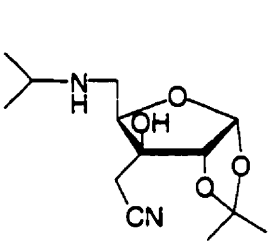


6-22

Résumé

L'auxiliaire chiral 1,2-di-O-isopropylidène-3-C-cyanoéthyl-5-déoxy-5-isopropylamino-D-xylofuranose **3-32** est synthétisé. La phosphoramidite cyclique **3-34** dérivée de **3-32** est un précurseur utile pour la synthèse stéréosélective de phosphorothioate. L'auxiliaire chiral peut être aisément enlevé à la fin de la synthèse par traitement à l'ammoniaque concentrée. L'isomère majeur de phosphorothioate de dithymidine de configuration S_P est obtenu.

Le groupe allyl fut étudié en tant que groupe protecteur pour la liaison internucleotide. Le clivage du groupe allyl des dimères **4-6S** où **4-6O** fut réalisé par des traitements nucléophiliques. Une étude en phase solide indique la généralité de cette méthode pour la synthèse de routine de phosphorothioates et de phosphates d'oligonucléotides en phase solide.



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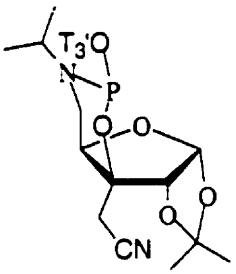

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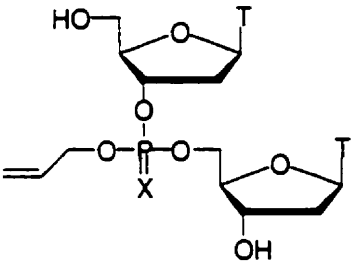
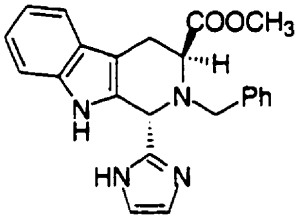


4-6 S X=S, **O** X=O

Un nouveau type d'auxiliaire chiral **5-20** qui incorpore des fonctions indole et imidazole est synthétisé. Il permet la synthèse stéréosélective d'un nouvel

indolophosphorothioate **5-37** avec un excès diastéréomérique de 90% et la formation d'un diastéromère unique d'un analogue d'alkylphosphinate **5-41**.

Le composé chiral indolo-oxazaphosphorine **6-22** est préparé à partir de L-tryptophane. Un phosphorothioate de dithymidine avec un excès diastéréomérique supérieur à 95% est obtenu en utilisant **6-22** en tant que précurseur. L'auxiliaire chiral est enlevé par thermolyse dans l'ammoniaque concentrée à 55 °C pendant 16 heures. Les indolo-oxazaphosphorines dérivées de L- où de D- tryptophane mènent à la formation de phosphorothioates de dithymidine avec des configurations R_P ou S_P, respectivement. La méthodologie est employée avec succès à la synthèse de dimères de phosphorothioate en phase solide.



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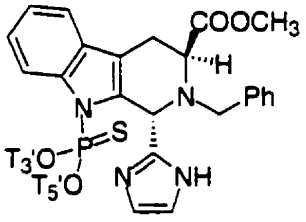

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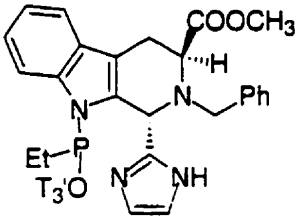

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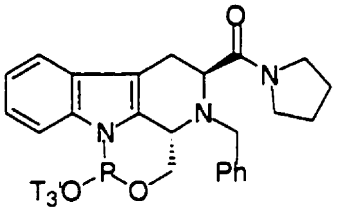

6-22

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My thanks also go to Ms. Renée Charron for her invaluable help in administrative matters. I am grateful to Chemistry Department, McGill University for funding in the form of teaching and research assistantships.

Glossary of Abbreviations

A	adenine
Ac	acetyl
AIDS	acquired immuno-deficiency syndrome
Ar	aryl
B	base
b.p.	boiling point
b	broad (NMR)
Bu	butyl
c	concentration (for the measurement of optical rotation)
C	cytosine
C	Celsius
Calc	calculated
CI	chemical ionization
COSY	COrrelation SpectroscopY
CPG	controlled pore glass
δ	chemical shift (NMR)
d	doublet (NMR)
dA	2'-deoxyadenosine
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
DCE	dichloroethane
dC	2'-deoxylcytidine
de	diastereomeric excess

DMAP	N,N-dimethyl-4-aminopyridine
DMF	dimthylformamide
DMSO	dimethylsulfoxide
DMTr	4,4'-dimethoxyltriphenylmethyl
DNA	deoxylribonucleic acid
dT	2'-deoxythymidine
E. coli	*Escherichia Coli*
EI	electronic ionization
eq.	equivalent
Et	ethyl
FAB	fast atom bombardment
g	gram
G	guanine
h	heptet (NMR)
h	hour
HCMV	cytomegalovirus (CMV) in HIV patient
HIV	human immunodeficiency virus
HMQC	heteronuclear multiple quantum correlation
HPLC	high performance liquid chromatography
HRMS	hugh resolution mass spectroscopy
Hz	Hertz
i-Pr	iso-propyl
J	coupling constant (NMR)

lit.	literature
µl	microliter
µmol	micromole
m	multiplet (NMR)
m	meta
MCPBA	meta-chloroperbenzoic acid
Me	methyl
mg	milligram
MHz	megaHertz
min	minute
mmol	millimole
mol	mole
MMTr	4,4'-dimethoxytriphenylmethyl
m.p.	melting point
mRNA	*messenger* ribonucleic acid
MS	mass spectroscopy
N	normal (solution)
NBA	nitrobenzyl alcohol
n-Bu	*n*-butyl
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
o-	ortho
p-	para

Ph	phenyl
ppm	parts per million
PSI	pounds per square inch (1 PSI = 0.06804 atm)
q	quartet (NMR)
R_f	retardation factor
RNA	ribonucleic acid
RT	room temperature
s	singlet (NMR)
sec	second
t	triplet (NMR)
T	thymidine
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
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
tRNA	transfer ribonucleic acid
Ts	tosyl (para-toluenesulfonyl)

U	uracil
UV	ultraviolet
v	volume
w	weight

Chapter 1. Introduction and Literature Survey

1.1. The Antisense Strategy

1.1.1. Principle of Antisense Strategy

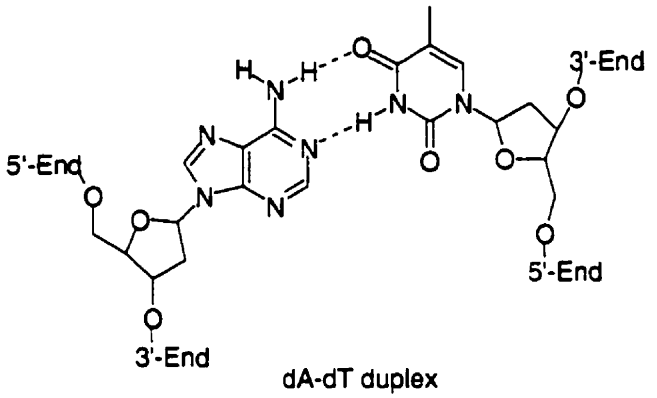
Traditionally, drug discovery involves the optimization of lead compounds which are derived from a painstaking process of synthesis and screening. A large number of compounds need to be synthesized in order to find something worth developing. Frequently, the active compound is directed against proteins such as enzymes, receptors, or ion channels, the structure and mode of action of which are usually very complicated. They often bind to non-target protein, leading to undesired side effects. Oligonucleotide based drugs, on the other hand, intervene earlier in the disease process – at the genetic level, by interrupting the process by which disease causing proteins are produced. Therefore, this approach is considered to be a route to rational drug design.

The principle is based upon Watson-Crick[1] hybridization of purine and pyrimidine bases as shown in Scheme 1.1, in which adenine recognizes specifically thymine, while guanine recognizes specifically cytosine.

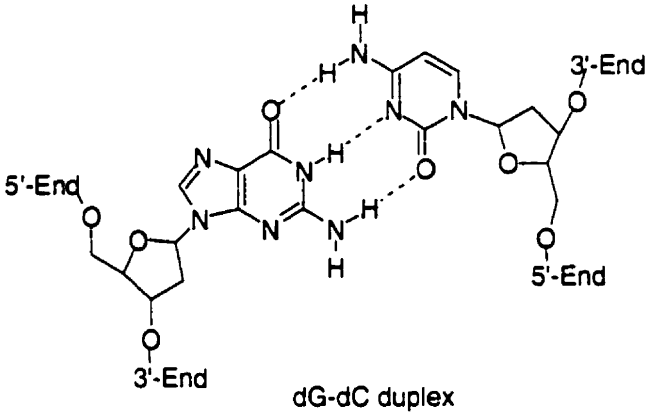
The use of synthetic oligonucleotides as a new class of potential therapeutics was first proposed by Zamecnik and Stephenson[2]. The excitement of the approach lies in that oligonucleotides may bind with high affinity and selectivity to their nucleic acid target, compared to the traditional drugs. In the antisense approach, oligonucleotides can interact with messenger RNA (mRNA) following the rules of Watson-Crick base paring. This

[1] Watson, J. D. and Crick, F. H. C. *Nature* **1953**, 171, 737

predictable way of interactions represents one of the most powerful systems for molecular recognition. On the other hand, synthesis of protein is also arrested when oligonucleotides bind to double-stranded DNA. This approach will not be discussed in this thesis.



dA-dT duplex



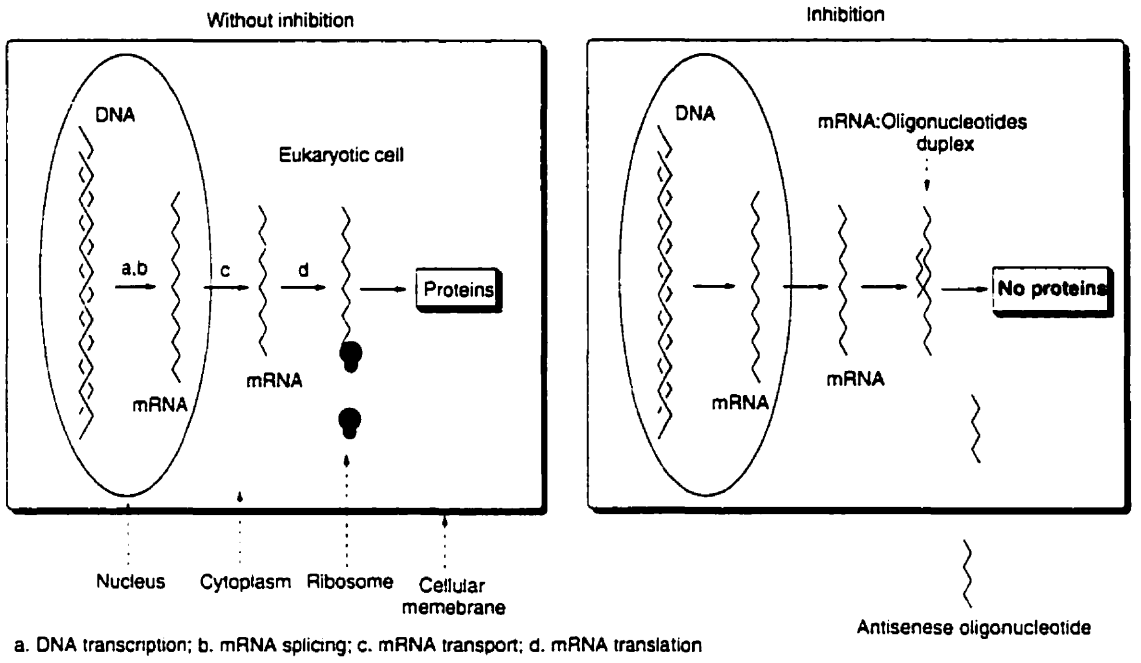
dG-dC duplex

Scheme 1.1: Watson-Crick base pairing

The original genetic information is contained in DNA. After transcription, the information in the cell is transcribed to mRNA and upon translation. amino acids will be assembled in certain ways to produce particular proteins. Antisense drugs are designed to

[2] Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, 75, 280

mirror specific (sense) mRNA sequences and block disease related protein production. If antisense drugs can penetrate into cells and bind specifically to mRNA to form a duplex, translation gets arrested, and therefore no proteins are produced (Scheme 1.2).



Scheme 1.2: Principle of the antisense strategy

1.1.2. Mechanisms of Action of Antisense Oligonucleotides

There are a number of complex mechanisms by which antisense oligonucleotides interact with nucleic acids.

(1) Inhibition of splicing

Oligonucleotides that bind to sequences required for splicing may result in inhibition of the production of the mature mRNA.[3]

(2) Translational arrest

[3] Kulka, M.; Smith, C. C.; Aurelian, L.; Fishelevich, R.; Meade, K.; Miller, P. and Ts'O, P. O. P. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, 86, 6868

Antisense oligonucleotides can form duplexes with mRNA at the point where translation is started. In this way important initiation factors may be prevented from binding to ribosomes, therefore inhibiting the translation of a nucleotide sequence into a peptidic sequence. A number of examples purporting to employ this mechanism have been reported.[4,5,6] This mechanism may represent an important mechanism of action for antisense drugs.

(3) Inhibition of transcription

Most antisense oligonucleotides were designed to inhibit translation. Only a few have been aimed at inhibiting transcription,[7,8,9] and none of them seem to have been successful.

(4) RNase H mechanism

RNase H is an ubiquitous enzyme that degrades the RNA strand of a RNA-DNA duplex.[10] Neither DNA-DNA nor RNA-RNA hybrids are attacked. RNase H has been identified in organisms as diverse as viruses and human cells.[11] When antisense oligonucloetides are targeted to mRNA and serve as substrate for RNase H, the cleavage of the mRNA induced by RNase H stops the transcription process. The precise recognition elements for RNase H remain unknown.

(5) Other mechanisms

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[4] Lemaitre, M.; Bayard, B. and Lebleu, B. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, 84, 648

[5] Vasanthakumar, G. and Ahmed, N. K. *Cancer Commun.* **1989**, 1, 225

[6] Mirabelli, C. K.; Bennett, C. F.; Andenson, K. and Crooke, S. T. *Anti-Cancer Drug Design* **1991**, 6, 647

[7] Cooney, M.; Czernuszewicz, G.; Postel, E. H.; Flint, S. J.; Hogan, M. E. *Science* **1988**, 241, 456

[8] Hélène, C. in *DNA-Ligand Interactions*; Guschlbauer, W.; Saenger, W.; Eds.; Plenum: New York, **1987**: pp.127

[9] Hélène, C.; Montenay-Garestier, T.; Saison, T.; Takasugi, M.; Toulmé, J. J.; Asseline, U.; Lanceolot, G.; Maurizot, J. C.; Toulmé, F.; Thuong, N. T. *Biochimie* **1985**, 67, 777

[10] Watson, J. D.; Hopkins, N. H.; Roberts, J. W.; Steitz, J. A.; Weiner, A. M. in *Molecular Biology of the Gene*, 4th ed. The Benjamin/Cummings publishing Company, Inc. **1987**, pp.298.

There are other mechanisms by which antisense oligonucleotides may work, such as: disruption of necessary RNA structure,[12,13] 5'-capping[14,15] or inhibition of 3'-polyadenylation.[16]

In conclusion, there are multiple mechanisms by which antisense oligonucleotides may operate. The mechanisms of action depend on the positions that are targeted, as well as the types of oligonucleotides used.

1.1.3. The Requirements for Antisense Drugs

In order to be used as drugs, antisense oligonucleotides have to meet certain requirements.

(1) Stability

Antisense oligonucleotides are designed to perform their therapeutic activity in the cell, so they have to be resistant to intracellular and extracellular enzymes.

(2) Cellular uptake

Antisense oligonucleotides should be able to be transported through cellular membranes, so that they can exert their biological functions.

(3) Affinity and specificity

One of the basic requirements to be met by antisense oligonucleotides is absolutely specific binding to the target nucleic acid. The affinity of oligonucleotides for

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[11] Crouch, R. J. and Dirksen, M. L.; Ribonucleases H. in *Nucleases*, eds. Linn S. M and Roberts, R. J., Cold Spring Harbor Laboratory press, Cold Spring Harbor, NY, **1985**, pp.211

[12] Vickers, T.; Baker, B. F.; Cook, P. D.; Zounes, M.; Buckheit, R. W., Jr.; Germany, J.; Ecker, D. J. *Nucleic Acids Res.* **1991**, 19, 3359

[13] Ecker, D. J.; Vickers, T. A.; Bruice, T. W.; Freier, S. M.; Jenison, R. D.; Manoharan, M.; Zounes, M. *Science* **1992**, 257, 958

[14] Baker, B. F.; Miraglia, L. and Hagedorn, C. H. *J. Biol. Chem.* **1992**, 267, 11495

[15] Baker, B. F. *J. Biol. Chem.* **1993**, 115, 3378

their targeting sequences results from hybridization interactions. The two major contributors are hydrogen bonding and base stacking in the double helix that is formed. Specificity derives from the selectivity of Watson-Crick base paring. Antisense oligonucleotides should clamp to the targeted region of RNA with high affinity and specificity.[17]

The length of oligonucleotides should also be considered. Oligonucleotides must be at least 17-18 nucleotides long in order to bind specifically to the target sites and avoid associating with non-target sites.[18] Longer than 25 bases may decrease uptake and efficacy of the oligonucleotide.[19]

(4) Low toxicity and cost

While maximally inhibiting production of disease-related proteins, antisense drugs should be non-toxic. Also, the drugs have to be available at a reasonable cost.

1.1.4. Chemical Modifications of Antisense Oligonucleotides

Naturally occurring oligonucleotides do not meet the criteria mentioned above as potential drug candidates due to their instability to nucleases and insufficient membrane penetration. Although natural oligonucleotides have displayed some activities *in vitro*,[20] they are degraded rapidly in animals.[21] Chemical modifications of oligonucleotides were proposed[22,23,24] to overcome the existing hurdles. The chemical modifications can be

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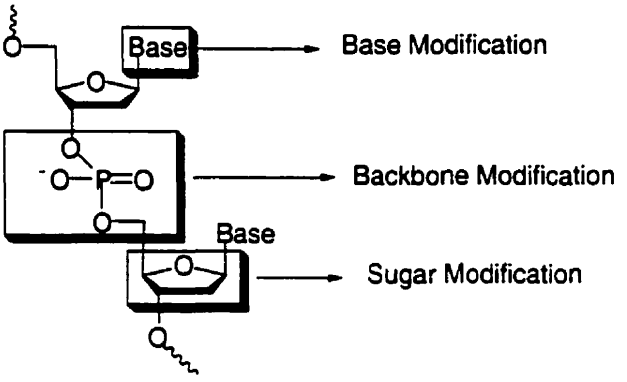
[16] Chiang, M. Y.; Chan, H.; Zounes, M. A.; Freier, S. M.; Lima, W. F. and Bennett, C. F. *J. Biol. Chem.* **1991**, 266, 18162
[17] Wagner, R. W. *Nature Medicine* **1995**, 5, 1116
[18] Cohen, J. S.; Hogan, M. E. *Sci. Am.* **1994**, 76
[19] Le Corre, S. M.; Burnet, P. W. J.; Meller, R.; Sharp, I.; Harrison, P. J. *Neurochem. Int.* **1997**, 31, 349
[20] Agrawal, S. *TIBTECH*. **1992**, 10, 152
[21] Agrawal, S.; Temsamani, J.; Galbraith, W.; Tang, J. *Clin. Pharmacokinet.* **1995**, 28, 7
[22] James, W. *Antiviral Chem.* **1981**, 2, 191
[23] Weintraub, H. M. *Sci. Am.* **1990**, 34

divided into three main categories: the modifications to the bases, to the sugar and to the phosphate backbone. (Scheme 1.3).



Scheme 1.3: Chemically modified oligonucleotides

Among the three categories of modifications, base modifications are limited[25,26,27,28,29,30,31] due to the necessity to maintain the Watson-Crick base pairing. Sugar modifications have been widely studied.[32] Up to the present time, the backbone modifications have been the most exploited and the most promising variations. As the hydrolytic cleavage of the phosphodiester backbone is the main cause for the rapid degradation of oligonucleotides by nucleases, their modifications are quite reasonable approaches to improve their stability. Backbone modifications retain the bases which are essential for binding and sequence specificity, and the sugar that allows for the

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[24] Goodchild, J. *Bioconjugate Chem.* **1990**, 1, 165
[25] Sanghvi, Y. S. in *Antisense Research and Applications*, Crooke, S. T.; Lebleu, B., Eds.; CRC press, Inc.; **1993**, pp.273-288
[26] Lin, K.; Jones, R. J.; Matteucci, M. *J. Am. Chem. Soc.* **1995**, 117, 3873
[27] Sanghvi., Y. S.; Hoke, G.D.; Freier, S. M.; Zounes, M. C.; Conzalex, C.; Cummins, L.; Sasmor, H.; Cook, P. D. *Nucleic Acids Res.* **1993**, 21, 3197
[28] Biala, E.; Jones, A. S.; Walker, R. T. *Tetrahedron* **1980**, 36, 155
[29] Froehler, B. C.; Wadwani, S.; Terhorst, T. J.; Gerrard, S. R. *Tetrahedron Lett.* **1992**, 33, 5307
[30] Hall, K. B.; McLaughlin, L. W. *Biochemistry* **1991**, 30, 1795
[31] Cheong, C.; Tinoco, I.; Chollet, A. *Nucleic Acids Res.* **1988**, 16, 5115
[32] For a review, see De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, 28, 366 and the references cited

orientation of the base with respect to the backbone axis. Backbone modifications can be sub-divided into the following categories.

(1) Oligonucleotides with modifications to the internucleotide phosphate residues

Backbone modifications that retain the phosphorus atoms are shown in Scheme 1.4: phosphorothioates[33,34](**a**), methylphosphonates[35](**b**), phosphoramidites[36,37,38](**c**), phosphotriesters[39](**d**), phosphorodithioates[40](**e**), boranophosphonates[41](**f**), phosphorofluoridites[42](**g**), phosphoroselenoates[43](**h**), phosphomorpholidates (**i**), phosphopiperazidates[44](**j**) and 4-pyridylphosphonates[45](**k**) have all been synthesized as modifications to the phosphodiesters. All the derivatives were shown to possess increased resistance towards nucleases. Phosphorothioates are the most well studied and most advanced antisense agents. The first antisense drug against CMV retinitis in the market – Vitravene™ (ISIS 2922) is a phosphorothioate which was developed by ISIS Pharmaceuticals.[46]

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[33] Eckstein, F. *Angewandte Chemie* **1983**, 22, 423

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[42] Dabkowski, W.; Cramer, F.; Michalski, J. *Tetrahedron lett.* **1987**, 28, 3561

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[44] Cazenave, C.; Hélène, C. in *Antisense Nucleic Acids and Proteins Fundamentals and Applications* Mol, J. N. M.; Van Der Krol, A. R. Eds,; Marcel Dekker, Inc. **1991**, pp.47

[45] Kers, A. and Stawinski, J. *Tetrahedron Lett.* **1999**, 40, 4263

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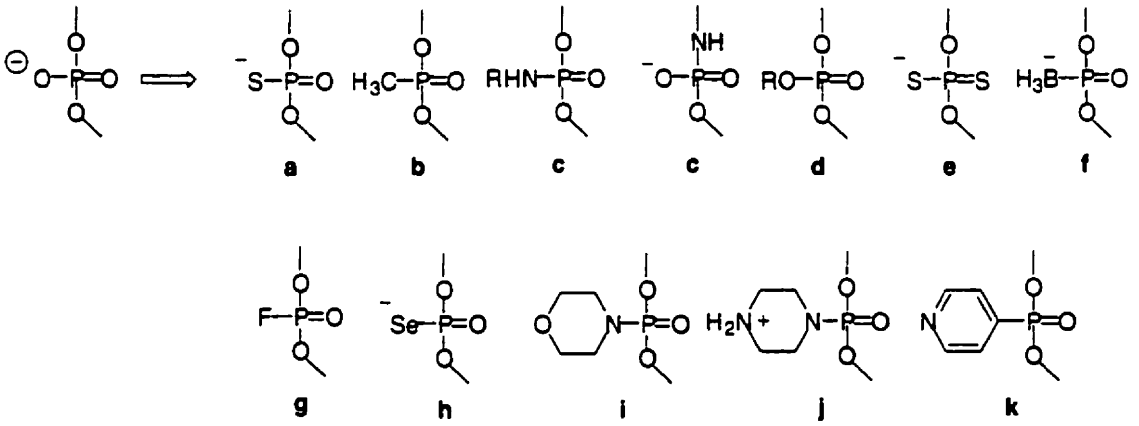

Scheme 1.4: Backbone modified DNA analogues

(2) Oligonucleotides with a linkage lacking phosphorus

Replacement of the phosphodiester linkage by different groups can obviously yield nonionic oligonucleotides. In the past decade, a number of articles were published[47,48,49,50] concerning this type of modification. Scheme 1.5 summarizes the "dephosphorus" modifications, including oligonucleotides containing siloxane[51,52](**l**), carbonate[53,54](**m**), carboxymethylene ester[55,56](**n**), amide[57,58](**o**), carbamate[59,60](**p**),

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[47] Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, 49, 6123
[48] Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, 36, 1923
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[55] Halford, M. H.; Jones, A. S. *Nature* (London) **1968**, 217, 638
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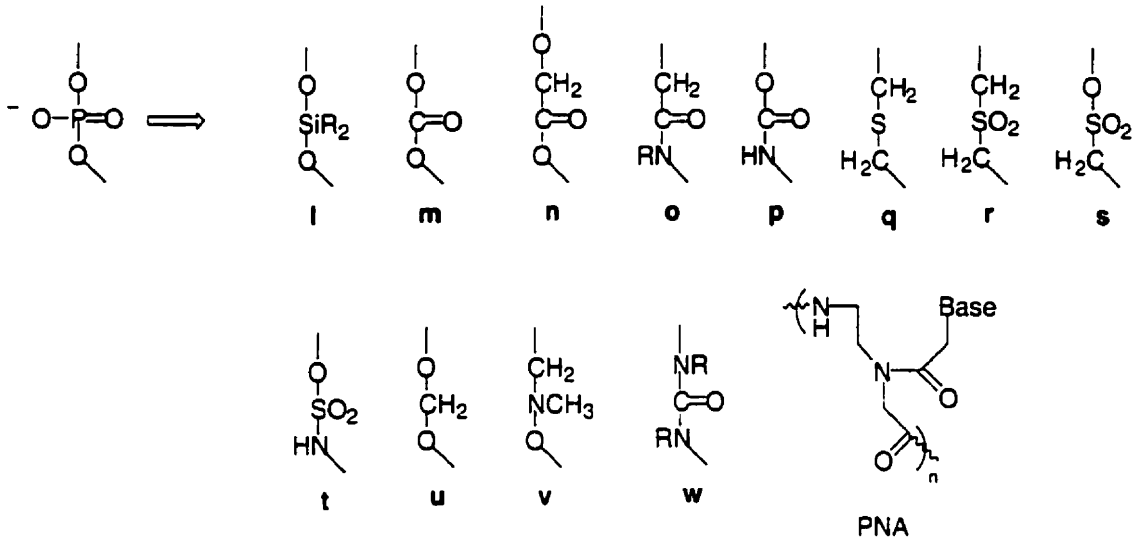
[58] Lebreton, J.; De Mesmeaker, A.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Tetrahedron Lett.* **1993**, 34, 6383
[59] Mungall, W. S.; Kaiser, J. K. *J. Org. Chem.* **1977**, 42, 703
[60] Coull, J. M.; Carlson, D. V.; Weith, H. L *Tetrahedron Lett.* **1987**, 28, 745

thioether[61](**q**), sulfone[62,63](**r**), sulfonate[64](**s**), sulfonamide[65](**t**), formacetal[66,67](**u**), methylene (methylimino) (MMI)[68](**v**) and urea[69] (**w**) bridges.



Scheme 1.5: "Dephosphorus" DNA analogues

Peptide nucleic acids[70,71,72](PNA) are very promising analogs of oligonucleotides with peptide bonds in the place of the sugar and phosphate backbone. It was reported that PNA binds strongly to both DNA and RNA[73]. In addition, they can be prepared on large scales at relatively low cost.

(3) The second generation antisense oligonucleotides

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[61] Kawai, S. H.; Just, G. *Nucleosides Nucleotides* **1991**, 10, 1485
[62] Schneider, K. C.; Benner, S. A. *Tetrahedron Lett.* **1990**, 31, 335
[63] Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org. Chem.* **1991**, 56, 3869
[64] Musicki, B.; Widlanski, T. S. *J. Org. Chem.* **1990**, 55, 4231
[65] Huie, A. M.; Kirshenbaum, M. R.; Trainor, G. L. *J. Org. Chem.* **1992**, 57, 4569
[66] Matteucci, M. *Tetrahedron Lett.* **1990**, 31, 2385
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[70] Egnolm, M.; Buchardt, O.; Nielson, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, 114, 1895
[71] Nielsen, P. E.; Egnolm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, 254, 1497
[72] Nielsen, P. E.; Egnolm, M.; Buchardt, O. *Bioconjugates Chem.* **1994**, 5, 3

Pure phosphorothioate oligonucleotides (PS-Oligos) have a number of undesired dose-dependent side effects, at least in animal studies.[74] In order to overcome these, and also improve the potency of phosphorothioates as antisense drugs, second generation oligonucleotides have been developed.

Chimeric or gapped oligonucleotides strategy[75,76] for oligonucleotide design is based upon biophysical, biochemical and biological properties of different P-modified oligonucleotides. For instance, the low affinity of PS-Oligos for mRNA target can be improved by incorporating an electronegative fluorine atom at the 2' position of the sugar.[77] Full 2'-O-F oligonucleotides do not activate RNase H. RNase H activity can be restored by inserting a phosphorothioate gap. Therefore, the hybrid oligonucleotides possess the ability to activate RNase H while at the same time having high affinity for mRNA. Except 2'-O-F modification, there are some other 2' modifications. 2'-O-propyl modification[78] not only has higher affinity for mRNA, but also provides some degree of nuclease resistance. 2'-O-methoxyethoxy modification is one of the best modifications.[79] It can improve nuclease resistance – incorporation of 2'-O-methoxyethoxy group appears to render a phosphodiester oligomer as nuclease resistant as a phosphorothioate oligomer. The affinity of an oligomer containing this modification for mRNA target is also enhanced. An aminopropoxy substitution at the 2'-position is another promising

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[73] Brown, S. C.; Thomson, S. A.; Veal, J. M.; Davis, D. G. *Science* **1994**, 265, 777

[74] Rawls, R. L. *C&N News* **1997**, 6, 35

[75] Inoue, H.; Hayase, Y.; Iwai, S. and Ohtsuke, E. *FEBS Lett* **1987**, 215, 327

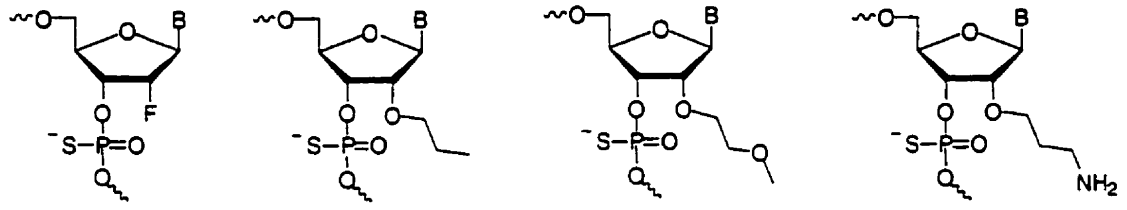
[76] Mckay, R. M.; Cummins, L. L.; Graham, M. J.; Lesnick, E. A.; Owens, S. R.; Winniman, M. and Dean, N. M. *Nucleic Acids Res.* **1996**, 24, 411

[77] Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzalez, C. and Cook, P. D. *J. Med. Chem.* **1993**, 36, 831

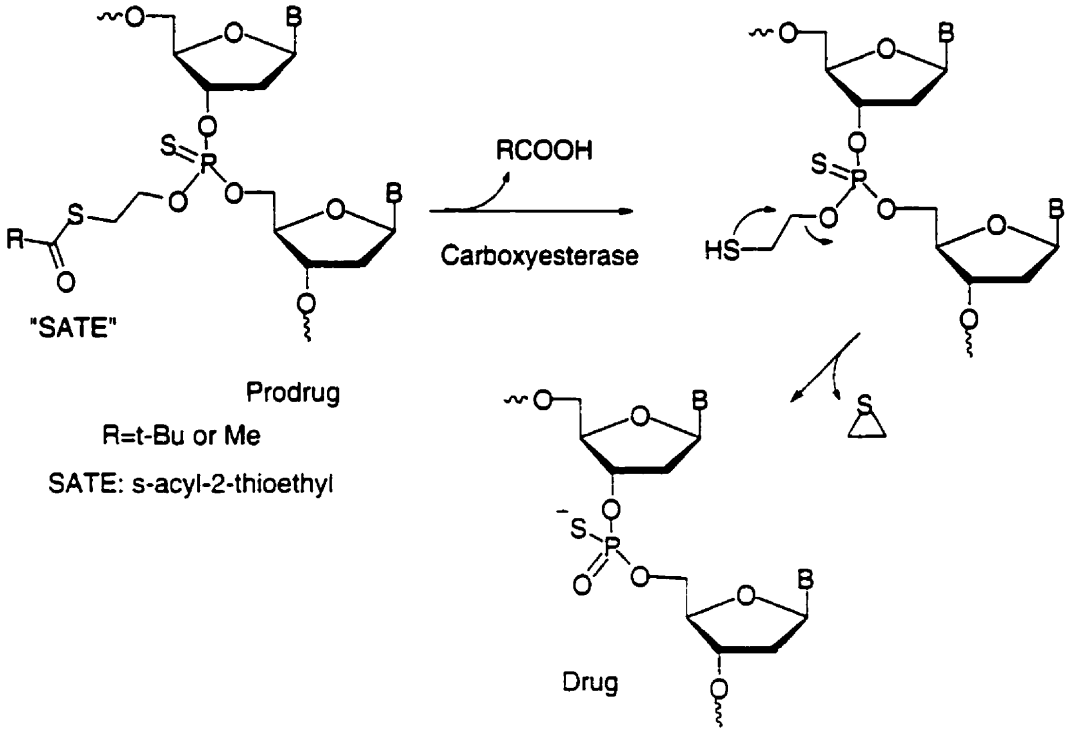
[78] Monia, B.; Johnston, J. F.; Sasmor, H. and Cummins, L. L. *J. Biol. Chem.* **1996**, 24, 14533

[79] Altmann, K.-H.; Dean, N. M.; Fabbro, D.; Freier, S. M.; Geiger, T.; Haner, R.; Husken, D.; Martin, P.; Monia, B. P.; Muller, M.; Natt, F.; Nicklin, P.; Phillips, J.; Pieles, U.; Sasmor, H. and Moser, H. E. *Chimia* **1996**, 50, 168

candidate.[74] Under physicological conditions, this moiety gets protonated, putting a positive charge in the minor groove of the DNA-RNA double helix which gives a tremendous increase in the nuclease resistance. Scheme 1.6 illustrates these 2'-O-modifications.



Scheme 1.6: Some 2' oligonucleotide modifications



Scheme 1.7: Prooligonucleotide approach

The polyanionic linkages of antisense oligonucleotides can be masked by "prodrug" approach. Imbach et al.[80] applied a prodrug approach which used an alkyl S-Acyl-Thio-Ethyl (alkyl-SATE) bioreversible protecting group to the delivery of 5'-mononucleosides. The general use of SATE prooligonucleotides is shown in Scheme 1.7. The neutral prodrug oligonucleotides could pass through cell walls easily, and subsequent removal of the SATE group can be achieved by a carboxyesterase mediated process.[81,82]

1.2. Phosphorothioate Oligonucleotides

1.2.1. The Application of Phosphorothioates as the Antisense Agents

Phosphorothioate oligonucleotides (PS-Oligos) have been the most well studied and most advanced antisense agents. The first synthesis of PS-Oligos dates back to 1969.[83] Replacement of one of the phosphate oxygen atoms in the bridge by a sulfur atom yields phosphorothioate with the negative charge being held mainly on the sulfur.[84,85]

PS-Oligos are nuclease resistant,[86,87,88,89] although not nuclease-proof. PS-Oligos are degraded slowly by cells in tissue culture with a half life of 12 to 24 hours and are

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[80] Perigaud, C.; Girardet, J.-L.; Gosselin, G.; Imbach, J.-L. *Adv. Antiviral Drug Des.* **1996**, 2, 147
[81] Mignet, N.; Morvan, F.; Rayner, B.; Imbach, J.-L. *Bioorg. Med. Chem. Lett.* **1997**, 7, 263
[82] Tosquellas, G.; Morvan, F.; Rayer, B.; Imbach, J.-L. *Bioorg. Med. Chem. Lett.* **1997**, 7, 851
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[84] Frey, P. A.; Sammons, R. D. *Science* **1985**, 228, 541
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[86] Campbell, J. M.; Bacon, T. A.; Wickstrom, E. J. *Biochem. Biophys. Methods* **1990**, 20, 259
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[88] Crooke, S. T. *Annu. Rev. Pharmacol. Toxicol.* **1992**, 32, 329
[89] Crooke, S. T. *Oligonucleotide Therapeutics* in Burger's Medicinal Chem. And Drug Discovery, Vol. 1 ed. M. E. Wolff, New York:Wiley and sons, Inc. pp.863

slowly metabolized in animals.[90,91] Moreover, PS-Oligos are more stable to various restriction endonucleases when in duplexes.

Although PS-Oligos are highly charged polyanionic molecules, replacement of an oxygen by sulfur confers on PS-Oligos more lipophilicity so that they have better cellular uptake.[92,93,94,95,96,97] PS-Oligos are taken up by a wide range of cells *in vitro*.[98,99,100] The uptake is time and temperature dependent. It is also influenced by cell type, cell-culture conditions, media and sequence and the length of the oligonucleotides. Several studies suggested that receptor-mediated endocytosis may be a significant mechanism of cellular uptake.[93]

One of the most important advantages of PS-Oligos is that they can elicit the activity of RNase H.[101] This enzyme, found predominantly in the cell nucleus, cleaves the RNA strand of a RNA-DNA duplex. The PS-Oligos thus can be thought of as a catalyst for mRNA cleavage. However, mRNA cleavage fragments from the cells targeted with antisense oligonucleotides are generally difficult to detect because of further rapid degradation.[102]

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[90] Crooke, S. T. *Therapeutic Applications of Oligonucleotides*, **1995**, Austin, R. G. Landes Company

[91] Cossum, P. A.; Sasmor, H.; Dellinger, D.; Truong, L.; Cummins, L.; Owens, S. R.; Markham, P. M.; Shea, J. P. and Crooke, S. T. *J. Pharm. Exp.Theraputics* **1993**, 267, 1181

[92] Yakubov, L. A.; Deeva, E. A.; Zarytova, V. F.; Ivanova, E. M.; Ryte, A. S.; Yurchenko, L. V.; Vlassov, V. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, 86, 6454

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[95] Stein, C. A.; Tonkinson, J. L.; Zhang, L.-M.; Yakubov, L.; Gervasoni, J.; Taub, R.; Rotenberg, S. A. *Biochemistry* **1993**, 32, 4855

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[97] Tao, I. F.; Marx, K. A.; Wongwit, W.; Jiang, Z.; Agrawal, S. and Coleman, R. M. *Antisense Res. Developments* **1995**, 5, 123

[98] Crooke, R. M. *Anti-Cancer Drug Des.* **1991**, 6, 609

[99] Gao, W. Y.; Han, F. S.; Storm, C.; Egan, W. and Cheng, Y. C. *Mol. Pharmacol* **1992**, 41, 223.

[100] Neckers, L. M. in *Antisense Research and Applications*, Eds. Crooke, S. T. and Lebleu, B., CRC press, Boca Raton, FL, **1993**, pp.451

[101] Mirabelli, C. K.; Bennett, C. F.; Anderson, K.; Crooke, S. T. *Anti-Cancer Drug Des.* **1991**, 6, 647

[102] Stein, C. A. *Chem.&Biol.* **1996**, 3, 319

The toxicity of PS-Oligos in human beings seems to be low. Hutcherson et al. reported[103] that when ISIS 2922 was administered against cytomegalovirus retinitis, the most common adverse event was anterior chamber inflammation which could be easily managed with steroids. When ISIS 2105 which was designed to inhibit the replications of human papilloma viruses that cause genital warts was administered, essentially no toxicities were observed.[104]

All the advantages mentioned above combined to make PS-Oligos extremely interesting for use in antisense technology. A number of PS-Oligos have entered into various stages of clinical trials against different diseases, including cancer and AIDS. Table 1.1 shows some examples.

Table 1.1: Some antisense PS-oligonucleotide drugs in clinical trials

Names	Gene Target	Sponsor	Diseases	Status
Gem 91[74]	HIV gag	Hydridon	AIDS	Phase II
GEM 132[74]	CMV	Hydridon	AIDS	Phase I
AR 177[105]	HIV integrase	Aronex	AIDS	Phase I
LR-3280[74]	c-myc	Lynx	Coronary Artery Disease	Phase I
GPI-2A[106]	HIV	Novopharm Biotech.	AIDS	Phase I
ISIS-2302[107,108,109]	ICAM-1	ISIS	Crohn's disease, Ulcerative Colitis, Rheumatoid Arthritis	Phase II
ISIS-5132[46]	c-raf kinase	ISIS	Cancer	Phase II
ISIS-5320[46]	HIV	ISIS	AIDS	Phase I

[103] Hutcherson, S. L.; Palestein, A. G.; Cantrill, H. L.; Liegerman, R. M.; Holland, G. N. and Anderson, K. P. *35th ICAAC* **1995**, 204

[104] Crooke, S. T. *Biotech. and Gene. Eng. Rev.* **1998**, 15, 121

[105] Ho, P. T. C.; Parkinson, D. R. *Semin. Oncol.* **1997**, 24, 187

[106] see webpage at http://www.novopharmbiotech.ca

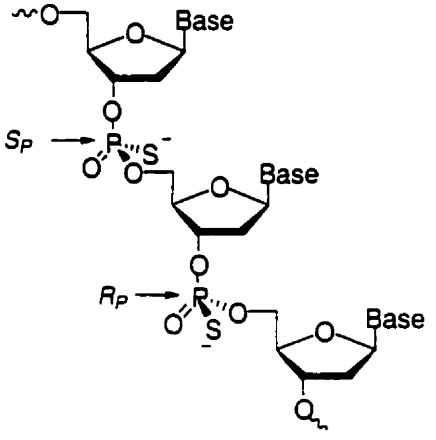
[107] Geary, R. S.; Leeds, J. M.; Henry, S. P.; Monteith, D. K.; Levin, A. A. *Anti-Cancer Drug Des.* **1997**, 12, 383

[108] Henry, S. P.; Monteith, D.; Levin, A. A.; *Anti-Cancer Drug Des.* **1997**, 12, 395

[109] Henry, S. P.; Taylor, J.; Midgley, L.; Levin, A. A.; Kornbrust, D. J. *Antisense Nucleic Acids Drug Dev.* **1997**, 7, 473

1.2.2. The Stereochemistry of Phosphorothioate Oligonucleotides

Structural modification by replacing one phosphate oxygen by sulfur has many advantages, as discussed above. However, it does create a stereochemical problem. As indicated in Scheme 1.8, there are four different groups attached to the phosphorus atom. Thus, a new chiral center is created. The chirality at the phosphorus center is designated as R_P or S_P. If non-stereoselective methods are applied to synthesize PS-Oligos, a large number of different diastereomers are obtained. Considering the number of the internucleotide linkage is n, then the total number of different diastereomers will equal to 2^{n-1}. For a 20-mer antisense PS-Oligos, the number of different diastereomers is 2^{19}. That is, more than half a million different compounds. This obviously is undesirable, especially when the mixture is used as a drug.



Scheme 1.8: Stereochemistry of phosphorothioates

Molecular recognition in biological systems usually depends on the stereochemistry of the molecules involved.[110] It is reasonable to think that only some of the diastereomers will have desirable physicochemical characteristics facilitating the most favorable biological effects.[111,112,113] Furthermore, different diastereomers would have different physico-chemical properties and consequently different biological properties. PS-Oligos possessing only R_P internucleotide linkages were found to be resistant to endonuclease P1, while oligonucleotides with S_P linkage were all cleaved under the same conditions.[114] Contrary to this, snake venom phosphodiesterase digested only terminal nucleotides having the R_P configuration.[115] Molecular modeling of phosphorothioates suggests a strong influence of phosphorus stereochemistry upon the hydration pattern and electrostatic potential surface of oligonucleotides.[116,117] Besides the concern of stereoselective synthesis of PS-Oligos from a theoretical and practical point of view, stereocontrolled synthesis of oligomers containing several chiral centers present a challenge for organic chemists. Stereoselective synthetic methods for phosphorothioate are summarized in the following section.

1.3. Synthesis of Phosphorothioate Oligonucleotides

1.3.1. Methods for Preparing Phosphorothioate Oligonucleotides

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[111] Zon, G. *Pharm. Res.* **1988**, 5, 539
[112] Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, 90, 544
[113] Miller, P. S. *Bio/Technolog* **1991**, 9, 358
[114] Griffiths, A. D.; Potter, B. V. L.; Eperon, I. C. *Nucleic Acids Res.* **1987**, 15, 4145
[115] Burgers, P. M. J.; Eckstein, F.; Hunneman, D. H. *J. Biol. Chem.* **1979**, 254, 7
[116] Hausheer, F. H.; Rao, B. G.; Saxe, J. D. and Singh, V. C. *J. Am. Chem. Soc.* **1992**, 114, 3201
[117] Sharp, K. A.; Honig, B. and Harvey, S. C. *Biochemistry* **1990**, 29, 340

PS-Oligos can be prepared routinely by modifications of the existing automated DNA synthesis technology.

(1) Historical overview of oligonucleotide synthesis

The first chemical synthesis of a dinucleotide was reported[118] by Michelson and Todd in 1955. Subsequently, Hall et al.[119] demonstrated that 2',3'-di-O-isopropylideneuridine-5'-hydrogen phosphonate was activated by diphenyl phosphochloridate to afford the corresponding dinucleoside hydrogen phosphate, which was easily oxidized to phosphodiester. In the late fifties and early sixties, Khorana[120,121] prepared short, defined sequences of deoxyribopolynucleotides using the "phosphodiester" approach. Letsinger and Ogilvie[122,123] introduced the so called "phosphotriester" approach which overcame the difficult separation problem faced in the "phosphodiester" approach. Letsinger et al.[124,125] also investigated the use of solid supports for the synthesis of oligonucleotides, which had been applied originally by Merrifield in the solid phase synthesis of peptides.[126]

A big advance for the chemical synthesis of oligonucleotide came in the mid-seventies when Letsinger and coworkers[127,128] developed the "phosphite triester" approach. The approach consisted of the reaction of a 5'-O-protected deoxythymidine with 2,2,2-trichloroethyl phosphorodichloridite to generate an intermediate

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[118] Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1955**, 2636
[119] Hall, R. H.; Todd, A.; Webb, R. F. *J. Chem. Soc.* **1957**, 3291
[120] Khorana, H. G. *Pure Appl. Chem.* **1968**, 17, 349
[121] Khorana, H. G. *Biochem. J.* **1968**, 109, 709
[122] Letsinger, R. L.; Ogilvie, K. K. *J. Am. Chem. Soc.* **1967**, 89, 4801
[123] Letsinger, R. L.; Ogilvie, K. K. *J. Am. Chem. Soc.* **1969**, 91, 3350
[124] Letsinger, R. L.; Mahadevan, V. *J. Am. Chem. Soc.* **1965**, 87, 3526
[125] Letsinger, R. L.; Mahadevan, V. *J. Am. Chem. Soc.* **1966**, 88, 5319
[126] Merrifield, R. B. *Science* **1965**, 150, 178
[127] Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, 98, 3655
[128] Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, 97, 3278

deoxyribonucleoside-3'-O-phosphorochloridite within 5 minutes at –78 °C. The addition of a 3'-O-protected deoxythymidine resulted in the rapid formation of the dinucleoside phosphite triester which was then oxidized to the corresponding phosphate triester.

Despite the popularity that the "phosphite triester" approach enjoyed, it did suffer from some shortcomings. The major one was that the highly reactive deoxyribonucleoside chlorophosphite intermediates were too moisture sensitive and thus very difficult to handle under normal conditions. In order to alleviate these problems, Beaucage and Caruthers[129] developed the phosphoramidite approach.

(2) The use of the phosphoramidite approach for the synthesis of phosphorothioates

The solid phase synthesis of PS-Oligos by the phosphoramidite approach is depicted in Scheme 1.9. In the phosphoramidite approach, the coupling reaction of the 5'-hydroxy group of a solid-supported growing DNA **1** with a cyanoethyl N, N-diisopropylphosphoramidite **2** is catalyzed by 1H-tetrazole to yield phosphite triester **3**. This is followed by sulfurization to provide phosphorothioate **4**, which can be deprotected to give 5'-hydroxy group. Repeating these sequences of reactions and removing the protecting groups in the end of the synthesis afford the final PS-Oligo **5**. Phosphoramidite intermediate **2** is less moisture sensitive and more easily handled than the chlorophosphite intermediate used in phosphite triester approach. The solid phase synthesis of PS-Oligos is easily performed. Fragments as long as 175 units have been prepared.[130]

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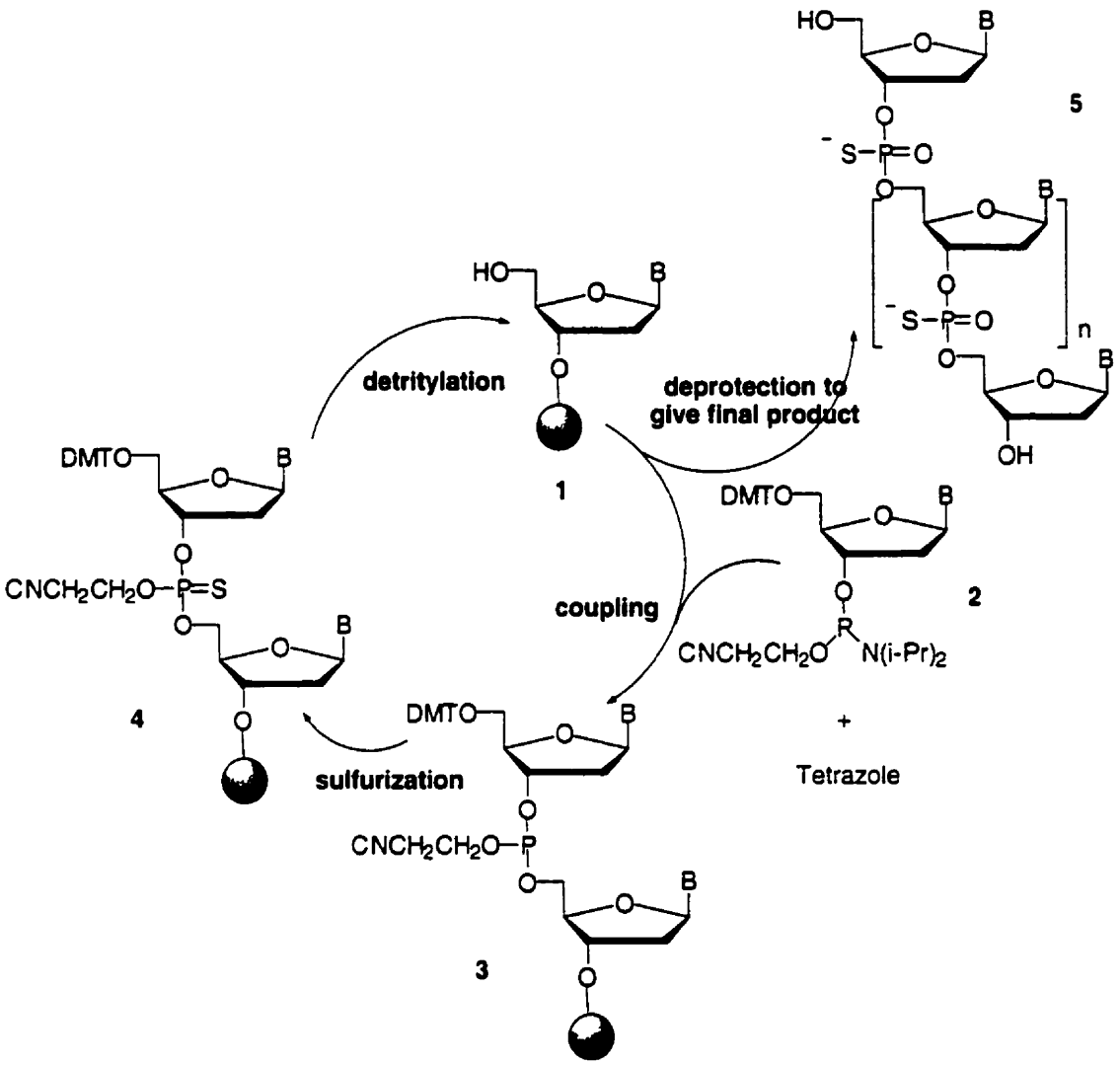
[129] Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, 22, 1859

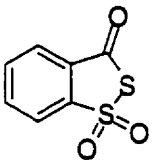
[130] Efcavitch, J. W,; McBride, L. J.; Eadie, J. S. in *Biophosphates and their Analogues/Synthsis, Structure, Metabolism and Activity:* Bruzik, K. S.; Stec, W. J., Eds., Elseviers Amsterdam, **1987**, pp.205



Scheme 1.9: Solid phase synthesis of PS-Oligos by phosphoramidite approach

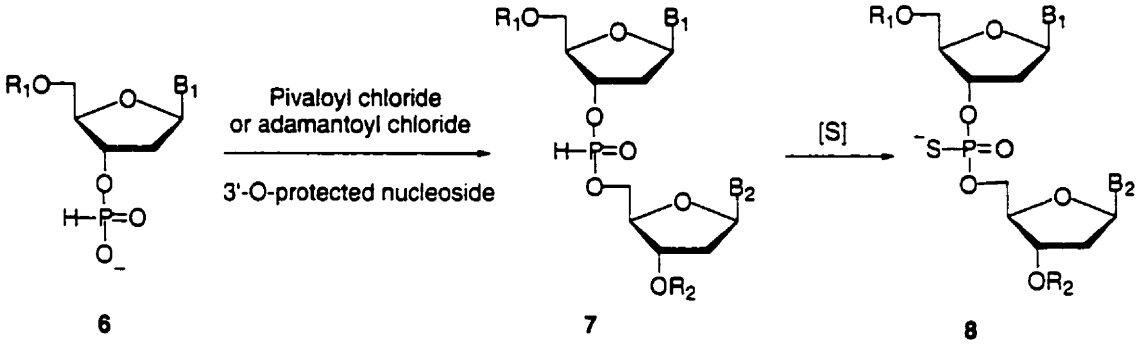
The reagents used for the sulfurization step are elemental sulfur (S_8) or Beaucage's sulfurizing reagent[131] (3H-1,2-benzodithiol-3-one 1,1-dioxide) (Scheme 1.10).

[131] Iyer, R. P.; Egan, W.; Ryan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, 112, 1253



Scheme 1.10: Beaucage's reagent

(3) The H-Phosphonate Approach



Scheme 1.11: Synthesis of a phosphorothioate from an H-phosphonate

Froehler et al.[132a,132b] first extended automated H-phosphonate chemistry to the synthesis of phosphothioate analogs. H-phosphonate **6**, activated by trimethylacetyl (pivaloyl) chloride or adamantoyl chloride, reacted with a 3'-O-protected nucleoside to give H-phosphonate **7** which upon sulfurization was transformed into phosphorothioate **8** (Scheme 1.11).

The general methods discussed above do not have stereocontrol at the phosphorus centers and therefore the resulting products are random mixtures of various

[132a] Froehler, B. C. *Tetrahedron Lett.* **1986**, 27, 5575
[132b] Froehler, B. C.; Matteucci, M. D. *Tetrahedron Lett.* **1986**, 27, 469

diastereomers. The stereocontrolled synthesis of PS-Oligos opens a new and exciting area for synthetic chemists to explore.

1.3.2. Stereoselective Synthesis of Phosphorothioate Oligonucleotides

One obvious and straightforward way of synthesizing phosphorothioates with well controlled stereochemistry at phosphorus centers is the separation of a mixture of diastereomeric oligonucleotides obtained by means of non-stereocontrolled methods. However, this method is very limited. The effectiveness of the separation depends on the length of the oligonucleotide chain. In practice, it is almost impossible to isolate tetramers as single diastereomers, let alone the longer ones. Development of the methods for the stereoselective synthesis of PS-Oligos becomes an important issue to be addressed. Up to date, there is no good method which allows the synthesis of PS-Oligos with well defined stereochemistry at each phosphorus center on a large scale. In what follows is a brief summary of the progress in this research area.

1.3.2.1. Enzymatic Method

The enzymatic synthesis of a polyribonucleotide containing a phosphorothioate linkage dates back to 1967. Eckstein and colleagues[133] used DNA-dependent RNA polymerase from *Escherichia Coli* (*E. Coli*) to synthesize a modified RNA copolymer in which adenine and udenine units would alternate and every other internucleotidic bridge would be a phosphorothioate. They later synthesized[134] polyribonucleotide containing an all phosphorothioate backbone using the same approach. The stereochemical course of

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[133] Matzura, H.; Eckstein, F. *Eur. J. Biochem.* **1968**, 63, 448
[134] Eckstein, F. and Gindl, H. *Eur. J. Biochem.* **1970**, 13, 558

polymerization was determined by the same group using 5'-O-(1-thiotriphosphate) as substrate.[135,136] The enzyme substrate was an S_P epimer and an inversion of configuration at the phosphorus center was observed, resulting in the phosphorothioate linkage having a R_P configuration.

It was found out that DNA-dependent DNA polymerase from *E. coli*,[137,138] phage T4,[139,140] phage T7,[141] *micrococcus luteus*,[142] polynucleotide phosphorylase,[143,144] tRNA nucleotidyl transferase,[145] RNA ligase,[146] and 2',5'-oligoadenylate synthetase[147] all can catalyze the formation of a 3'-5' or 2'-5' internucleotide phosphorothioate linkage, always with R_P configuration at the phosphorus centers.

Generally speaking, the enzymatic synthesis of PS-Oligos is limited to short sequences and suffers from low yield. In addition, it is also impossible to obtain PS-Oligos having internucleotide linkages with an S_P configuration.

1.3.2.2. The Oxathiaphospholane Method

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[135] Eckstein, F.; Armstrong, V. W. and Sternbach, H. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, 73, 2987
[136] Burgers, P. M. J. and Eckstein, F. . *Proc. Natl. Acad. Sci. U. S. A.* **1978**, 75, 4798
[137] Burgers, P. M. J.; Eckstein, F. *J. Biol.Chem.***1979**, 254, 6889
[138] Brody, R. S.; Frey, P. A. *Biochemistry* **1981**, 20, 1245
[139] Romaniuk, P. J.; Eckstein, F. *J. Biol. Chem.* **1982**, 257, 7684
[140] Gupta, A.; DeBrosse, C.; Benkovic, S. *J. Biol. Chem.* **1982**, 257, 7689
[141] Brody, R. S.; Adler, S.; Modrich, P.; Stec, W. J.; Lesnikowski, Z. J.; Frey, P. A. *Biochemistry* **1982**, 21, 2570
[142] Eckstein, F.; Jovin, T. M. *Biochemistry***1983**, 22, 4546
[143] Burgers, P. M. J.; Eckstein, F. *Biochemistry* **1979**, 18, 450
[144] Marlier, J. F.; Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1981**, 20, 2212
[145] Eckstein, F.; Sternbach, H.; von der Haar, F. *Biochemistry* **1977**, 16, 3429
[146] Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1982**, 21, 5877
[147] Suhadolnik, R. J.; Choongeun, L. *Biochemistry* **1985**, 24, 551

The most advanced method for stereoselective synthesis of PS-Oligos to date is the oxathiaphospholane method[148,149,150,151,152,153] developed by Stec and coworkers (Scheme 1.12). In this method, the starting oxathiaphospholanes **10** or **11** were first prepared as a pair of diastereomers. They were then separated by chromatography. Each isomer was reacted with nucleoside **9** which was bound to the solid support at the 3'-position. Intermediates **12** or **13** were formed accordingly. Subsequently spontaneous loss of episulfide leads to the formation of the internucleotide phosphorothioate function in over 95% yield and over 98% diastereomeric excess (de). After repetition of the coupling cycles, stereochemically defined PS-Oligos can be obtained.

However, there are still some weak points in this approach. The column chromatographic separation of the starting oxathiaphospholane diastereomeric mixture is very difficult and also accompanied by the loss of a large amount of precursors **10** or **11** as reported by Stec et al.[154] In addition, the condensation step required large excess of DBU and precursors **10** or **11**, which made this method not applicable to large scale preparation.

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[148] Stec, W. J.; Okruszek, A.; Lesiak, K.; Uznanski, B. and Michalski, J. *J. Org. Chem.* **1976**, 41, 227

[149] Stec, W. J. *Acc. Chem. Res.* **1983**, 16, 411

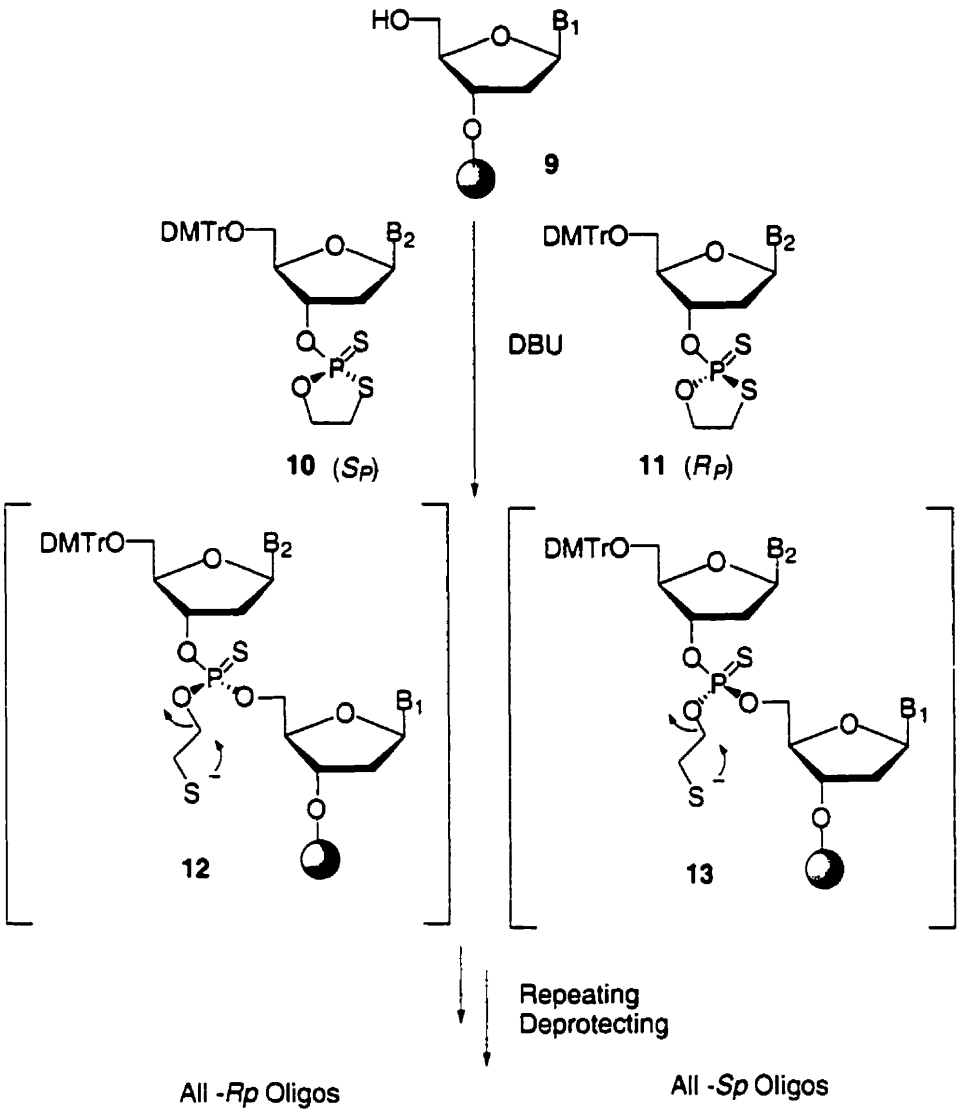
[150] Lesnikowski, Z. J.; Niewiarowski, W.; Zielinski, W. S. and Stec, W. J. *Tetrahedron* **1984**, 40, 15

[151] Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M. and Uznanski, B. *Nucleic Acids Res.* **1991**, 19, 5883

[152] Suska, A.; Grajkowski, A.; Wilk, A.; Uznanski, B.; Blaszczyk, J.; Wieczorek, M.; Stec, W. J. *Pure Appl. Chem.* **1993**, 65, 707

[153] Stec, W. J.; Wilk, A. *Angew. Chem. Intl. Ed. Engl.* **1994**, 33, 709

[154] Stec, W. J.; Grajkowski, A.; Kobylanska, A.; Karwowski, B.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, 117, 12019



Scheme 1.12: Stec's oxathiaphospholane method

1.3.2.3. Other Methods

As discussed in the previous section, phosphorothioates can be prepared by sulfurization of H-phosphonate precursor. Furthermore, sulfurization of the

diastereomerically pure dinucleotide H-phosphonate is known[155,156,157] to be a stereoretentive process. Therefore, stereocontrolled synthesis of the H-phosphonates can serve as a convenient precursor for stereocontrolled synthesis of phosphorothioates.

The diastereomerically pure H-phosphonates could be obtained by chromatographic separation of the two diastereomers formed during non-stereoselective reactions,[158,159,160] which were then transformed diastereospecifically into phosphorothioates. But this method is obviously not practical for a large scale preparation of stereochemically defined PS-Oligos. Clearly there exists a need to obtain diastereomerically pure H-phosphonates during stereoselective reactions instead of the tedious separation of the two isomers.

There have been only two examples in the literature[161,162,163] using H-phosphonate approach in which the stereochemistry at the phosphorus atom was generated during the formation of the H-phosphonate moiety. However, the two approaches were only limited to the synthesis of dimers. They did not seem to be applicable to the synthesis of longer oligomers and no further studies were reported.

Apart from the methods that have been discussed, there are some other methods available. [164,165,166,167] None of them seems to be a general method for the stereoselective synthesis of PS-Oligos.

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[155] Seela, F.; Kretschmer, U. *J. Chem. Soc. Chem. Com.* **1990**, 1154
[156] Szafraniec, L. J.; Szafraniec, L. L.; Aaron, H. S.; *J. Org. Chem.* **1982**, 47, 1936
[157] Stec, W. J.; Okruszek, A.; Michalski, J. *Angew. Chem.* **1971**, 83, 491
[158] Seela, F.; Kretschmer, U. *Nucleosides Nucleotides* **1991**, 10, 711
[159] Stawinski, J.; Stromberg, R.; Zain, R. *Tetrahedron Lett.* **1992**, 33, 3185
[160] Almer, H.; Stawinski, J.; Stromberg, R.; Thelin, M. *J. Org. Chem.* **1992**, 57, 6163
[161] Fujii, M.; Ozaki, K.; Kume, A.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1986**, 26, 935
[162] Fujii, M.; Ozaki, K.; Sekine, M.; Hata, T. *Tetrahedron* **1987**, 43, 3395
[163] Battistini, C.; Brasca, M. G.; Fustinoni, S.; Lazzari, E. *Tetrahedron* **1992**, 48, 3209
[164] Crosstick, R.; Williams, D. M. *Nucleic Acids Res.* **1987**, 15, 9921
[165] Lesnikowski, Z. J.; Sibinska, A. *Tetrahedron* **1986**, 42, 5025

1.3.2.5. Cyclic Phosphoramidite Approach

In our research group, we have been involved for some time in synthesizing diastereomerically pure P-chiral DNA analogs, particularly phosphorothioates. In what follows is a brief summary of the work which has been done in our laboratory.

Using the well-established phosphoramidite approach, two problems need to be solved in order to adapt it to a stereospecific synthesis of phosphorothioates. The first one is how to obtain chiral phosphoramidite without tedious column chromatographic separation of the two isomers. The second one would be, upon obtaining the chiral phosphoramidite, how to transform it in a stereospecific manner into a phosphite triester.

Xin and Just[168,169] were the first ones to use the cyclic phosphoramidite approach to obtain chiral phosphoramidites. As indicated in Scheme 1.13, reaction of chiral γ-aminoalcohol **14** with phosphorus trichloride in the presence of triethylamine gave a single diastereomer **15** with a single ^{31}P NMR resonance at 160.6 ppm. Without purification, further reaction with 5'-O-TBDMS thymidine (T_3-OH) and triethylamine yielded phosphoramidites **16a** and **16b** in a ratio of 3:1. The ratio was improved to 20:1 upon heating the mixture to reflux to equilibrate the two isomers. After passing through a short column, phosphoramidite **16a** was obtained as a single diastereomer. The next challenging problem would be transforming the chiral phosphoramidite into phosphite triester with well controlled stereochemistry.

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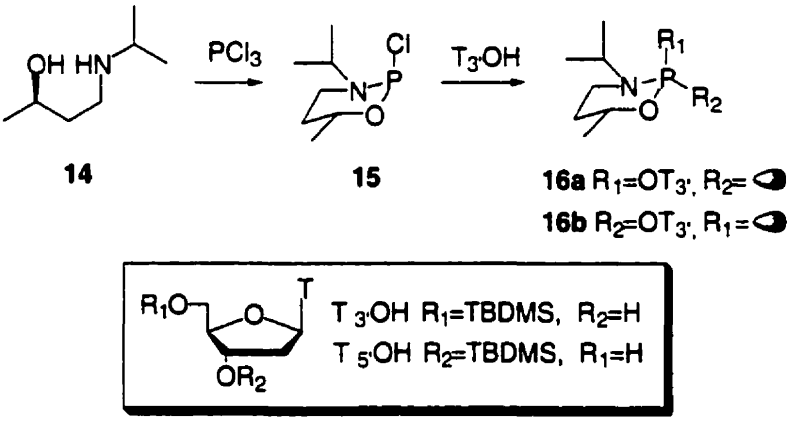
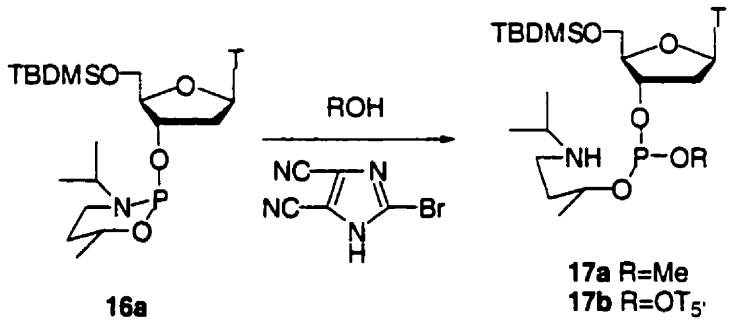
[166] Lesnikowski, Z. J.; Sibinska, A.; Jaworska, M. *Tetrhedron Lett.* **1989**, 30, 3821
[167] Wada, T.; Kobayashi, N.; Sekine, M. T. *Nucleosides Nucleotides* **1998**, 17, 351
[168] Xin, Z. and Just, G. *Tetrahedron Lett.* **1996**, 37, 969
[169] Xin Zhili, Master's thesis, McGill University, **1994**



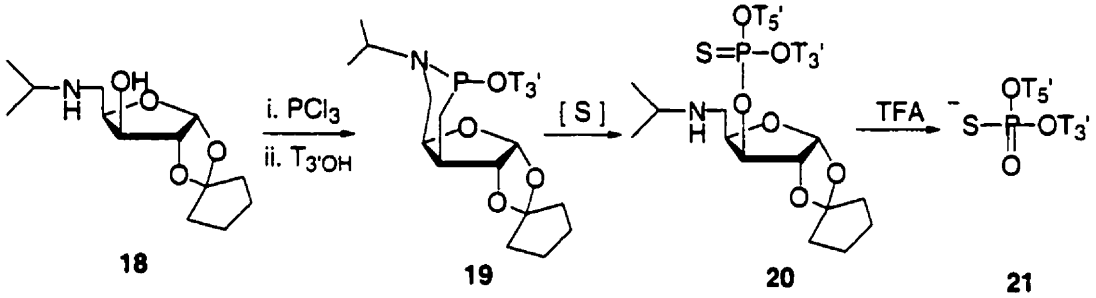
Scheme 1.13: First cyclic phosphoramidite

In the coupling reaction, chiral phosphoramidite was reacted with an alcohol by using tetrazole as catalyst. The phosphite triester was obtained with complete loss of stereochemistry as had been demonstrated by Stec et al.[170] for the acyclic phosphoramidite. Xin and Just then investigated the roles of various triazoles and imidazole as catalysts in the coupling reactions. Among them, the best catalyst was 2-bromo-4,5-dicyanoimidazole. As outlined in Scheme 1.14, phosphoramidite **16a** was reacted with alcohols in the presence of 2-bromo-4,5-dicyanoimidazole. When methanol was used as an alcohol, two diastereomers of phosphite triesters **17a** were obtained in a ratio of 50 to 1. This was a very exciting result. However, when T_3'OH was used, the ratio of the two diastereomers of **17b** dropped dramatically to 3 to 1. It was unclear whether the stereoselectivity was due to the size or the acidity of the 2-bromo-4,5-dicyanoimidazole.

[170] Stec, W. J. and Zon, G. *Tetrahedron Lett.* **1984**, 25, 5279.



Scheme 1.14: Coupling reactions with alcohols

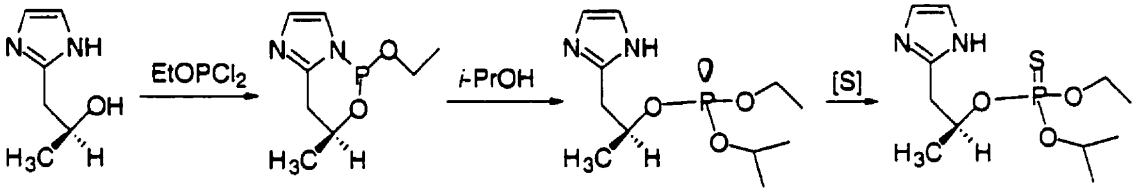


Scheme 1.15

The chiral auxiliary **14** did not give satisfactory stereoselectivity, and it was also not removable at the end of the synthesis. 1,2-Di-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **18** was then synthesized by Jin, Biancotto and Just[171] and used for the stereoselective synthesis of phosphorothioates. As shown in Scheme 1.15, chiral auxiliary **18** was first transformed to phosphoramidite **19**. Upon heating at 50 °C overnight, **19** was obtained as a single diastereomer, which had a ^{31}P NMR resonance at 130.1 ppm. When the coupling reaction was carried out in $CDCl_3$ at –15 °C, using 2-bromo-4,5-dicyanoimidazole as the catalyst, phosphite triester and phosphorothioate

[171] Jin, Y.; Biancotto, G. and Just, G. *Tetrahedron Lett.* **1996**, 37, 973

could be obtained with a diastereomeric ratio of 70 to 1. The removal of the chiral auxiliary was achieved by treatment with 70% aqueous trifluoroacetic acid solution. Nevertheless, this method also has its disadvantages. The catalyst 2-bromo-4,5-dicyanoimidazole was too acidic to be compatible with the dimethoxytrityl protecting group which is typically used for solid phase synthesis. The aqueous trifluoroacetic acid might cause depurination when bases other than thymidine are used, and the coupling temperature is obviously too low for a routine solid phase synthesis.



22 **23** **24** **25**

Scheme 1.16: Imidazo-oxazaphosphorine approach

It was then reasoned that if the catalyst could incorporate into a ring structure as shown (**23**), alcohol could attack it directly to form a phosphite triester, thus eliminating the use of acidic catalyst. No epimerization should therefore occur. Marsault and Just[172,173,174] then tried to incorporate imidazole into the chiral auxiliary. As indicated in Scheme 1.16, chiral auxiliary **22** which contained imidazole was transformed to imidazo-oxazaphosphorine **23** which had a single ^{31}P NMR resonance at 118.3 ppm. Cyclic **23** was too reactive to be isolated, and was heated with isopropanol to yield phosphite triester **24** with typical phosphite triester resonance at 140.6 ppm. Sulfurization gave

[172] Marsault, E. and Just, G. *Tetrahedron Lett.* **1996**, 37, 977

phosphorothioate **25** as a single diastereomer. However, when they tried to install a nucleoside as the exocyclic phosphorus substituent in order to carry out the displacement of imidazo-oxazaphosphorine, it turned out to be extremely difficult and impractical. This approach was then abandoned. But this work did prove that the displacement of the imidazole moiety in the imidazo-oxazaphosphorine intermediate was a stereospecific process. This method is good for the synthesis of the simple chiral phosphite triesters and the corresponding phosphorothioates.

Since the intermediate containing imidazole was too labile, indole was then investigated as a more stable leaving group.[175,176] In indolo-oxazaphosphorine approach (Scheme 1.17), chiral auxiliary **26** containing an indole moiety was reacted with phosphorus trichloride first, then with T_3-OH to produce **27a** and **27b** having ^{31}P NMR resonances at 120.53 ppm and 120.72 ppm respectively, in a ratio of 12 to 1. When treated with 3'-O-TBDMS thymidine (T_5-OH) and DBU, the major isomer **27a** reacted much faster to yield only one diastereomer of **28**. Sulfurization gave phosphorothioate **29**. The chiral auxiliary could be removed easily with concentrated aqueous ammonia to yield the desired phosphorothioate dimer **30**. This methodology worked very well in the solution phase.

However, when it was applied to solid phase synthesis, a rearrangement occurred to give an alkyl phosphonate as the major product instead of a phosphorothioate (Scheme 1.18). It was postulated[177] that the β-elimination of **27a** gave **31**, which upon reaction

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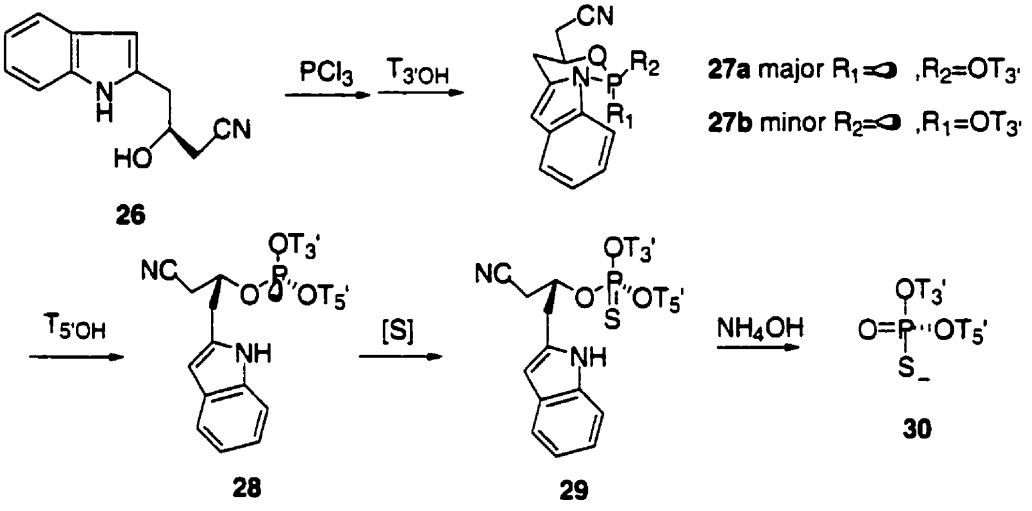
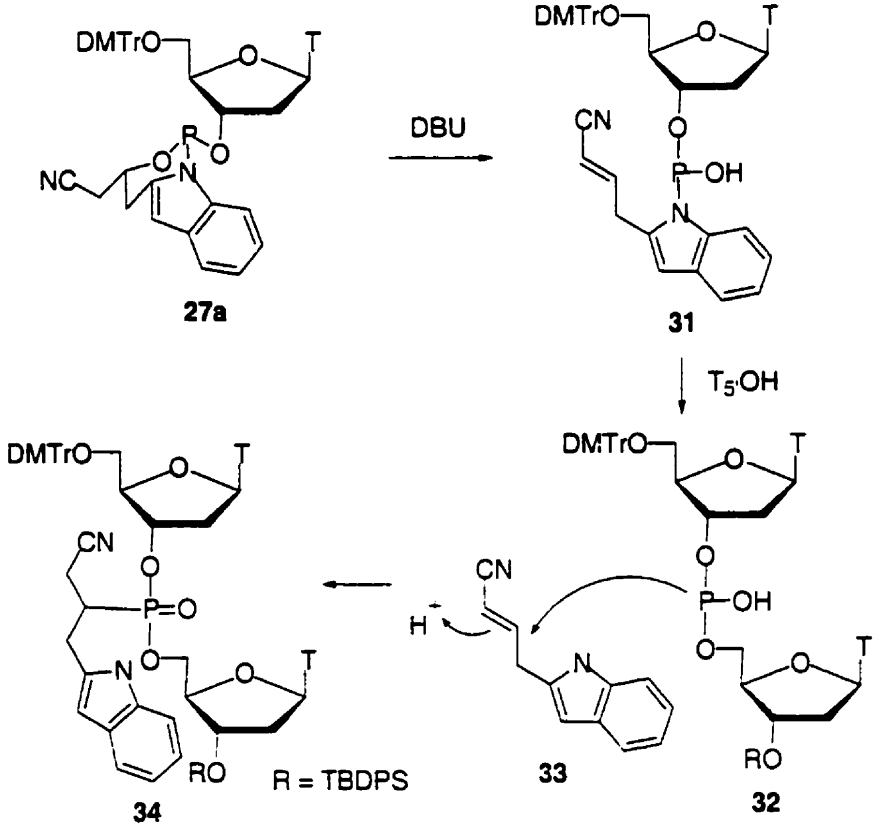
[173] Marsault, E. Ph.D. thesis, McGill University, **1996**
[174] Marsault, E. and Just, G. *Nucleosides Nucleotides* **1998**, 17, 939
[175] Wang, J. and Just, G. *Tetrahedron Lett.* **1997**, 38, 705
[176] Wang, J. and Just, G. *Tetrahedron Lett.* **1997**, 38, 3797
[177] Wang, J.; Just, G.; Guzaev, A. P.; Manoharan, M. *J. Org. Chem.* **1999**, 24, 2595

with another thymidine was transformed into **32**. The latter then added to the α,β-unsaturated nitrile **33** to give alkylphosphonate **34**.



Scheme 1.17: Indol-oxazaphosphorine approach



Scheme 1.18: The formation of alkylphosphonate

In the xylose based cyclic phosphoramidite approach, a reasonable degree of stereoselectivity was achieved when 2-bromo-4,5-dicyanoimidazole was used as the catalyst in the coupling reaction. We therefore also synthesized catalysts larger than the former in an effort to improve the stereoselectivity. This work is discussed in Chapter 2.

The use of 1,2-di-O-cyclopentylidene-5-deoxy-5-isoproylamino-α-D-xylofuranose **18** as chiral auxiliary led to the highly stereoselective formation of phosphorothioate. However, its removal was only achieved by treatment with 70% aqueous trifluoroacetic acid. In order to easily remove the chiral auxiliary, we modified it by forming a tertiary alcohol at the 3-position of xylose. Chapter 3 summarizes this investigation.

Our interest in developing an easily removable protecting group for the internucleotide linkage was first initiated by the work on formation of tertiary alcohol for the removal of the chiral auxiliary. We then developed the facile nucleophilic removal of allyl protecting groups for the internucleotide phosphates and phosphorothioates, which are described in Chapter 4.

In the imidazo-oxazaphosphorine approach (Scheme 1.16), the displacement of the imidazole moiety in imidazophoshorine **23** by alcohols occurred very rapidly in a stereospecific manner. As illustrated in the indolo-oxazaphosphorine approach (Scheme 1.17), the indole residue in indolophosphorine **27** could be displaced stereospecifically by 5'-hydroxy thymidine in the presence of DBU. We therefore tried to combine both leaving groups to develop a recoverable chiral auxiliary. This investigation is discussed in Chapter 5.

Derived from natural L- or D- tryptophan, we synthesized a new type of chiral indolo-oxazaphosphorine. The use of the chiral auxiliaries led to the stereoselective synthesis of dithymidine phosphorothioates in both solution and solid phases. These results are discussed in Chapter 6.

Chapter 2. Development of Catalysts for the Stereoselective Synthesis of Phosphite Triesters

2.1. Proposed Epimerization Mechanism

Oligonucleotides are most commonly prepared on a solid support using the phosphoramidite method developed by Caruthers and coworkers[129,178] (Scheme 1.9).

In order to address the stereochemistry of the P-chiral center in DNA analogues, it seemed reasonable to separate the two diastereomers of the phosphoramidite, and react one single diastereomer with a nucleoside to obtain a chiral phosphite triester. This work was reported by Stec and coworkers.[179] However, what they observed was the complete epimerization at the phosphorus center. The mechanism proposed by them is shown in Scheme 2.1. The first step was the displacement of the protonated amino group by tetrazole, followed by rapid displacement of the tetrazole group by another tetrazole. Upon slow reaction with alcohols, two epimers were obtained instead of one.

According to this mechanism, if we can have a bigger catalyst than tetrazole, it is reasonable to expect the epimerization process would be slowed down, and therefore a better selectivity could be obtained. In our research group, Zhili Xin[169] has tested various acidic catalysts in the activation of the amino part of the phosphoramidite in the coupling reactions. The most effective catalyst she found was 2-bromo-4,5-dicyanoimidazole. We therefore decided to synthesize catalysts larger than the former and see if they can give better selectivity.

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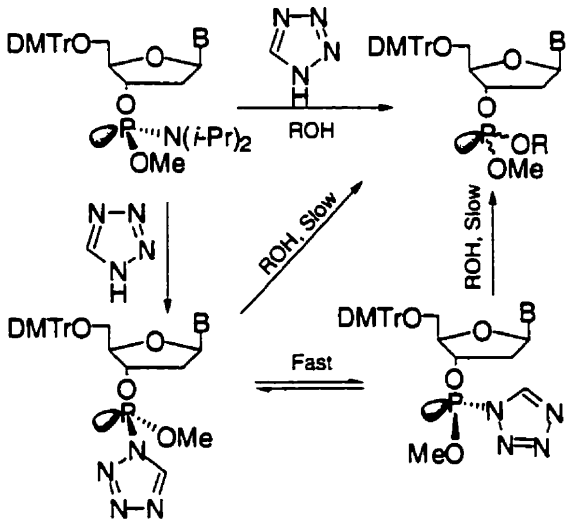
[178] McBride, L.J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, 24, 245



Scheme 2.1: Proposed epimerization mechanism

2.2. Synthesis of the Catalysts

In our research group, Yi Jin[180] tried unsuccessfully to install ester instead of cyano groups in the catalyst. We decided to keep cyano groups at the 4 and 5 positions of imidazole to confer the necessary acidity and install groups bigger than bromo at the 2-position. 2-Iodo-4,5-dicyanoimidazole seemed to be a good candidate. We also wanted to put a more electron withdrawing group, or much more hindered aromatic ring, at the 2-positions and find out their effects on the diastereoselectivities in the coupling reactions.

2.2.1. Through the Protection of 1-Position of Imidazole

Since 4,5-dicyanoimidazole is commercially available, one obvious approach for making the catalysts is to protect the 1-position of imidazole, stripping off the proton at the 2-position and quenching with various electrophiles (Scheme 2.2).

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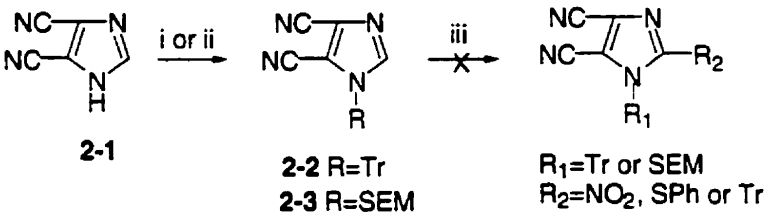
[179] Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, 25, 5279

[180] Jin, Y. and Just, G. *Private communication.*



2-1

2-2 R=Tr
2-3 R=SEM

R_1=Tr or SEM
R_2=NO_2, SPh or Tr

i. TrCl, Et_3N, CH_2Cl_2; ii. NaH, SEMCl iii. *n*-BuLi followed by $Ph_3C^+BF_4^-$ or PhSSPh or $NO_2^+BF_4^-$

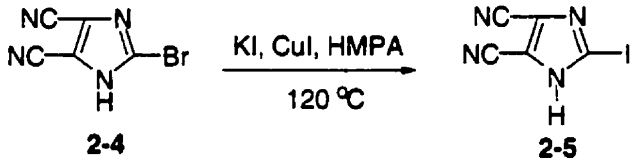
Scheme 2.2: Protecting imidazole at 1-position

4,5-Dicyanoimidazole **2-1** was first protected with a trityl group at the 1-position to give N-trityl-4,5-dicyanoimidazole **2-2**. It was then reacted with *n*-butyllithium to form the anion first. Various electrophiles were then added to the anion solution. In all cases, TLC showed complicated mixtures, and no major product was formed. It seemed that the 2-position might be too hindered by the neighboring trityl group for electrophiles to approach. Therefore, the 2-(trimethylsilyl)ethoxymethyl (SEM) group was introduced as an alternative protecting group to afford compound **2-3**. However, the reactions virtually went the same way. Suspecting anion formation was not complete, we treated **2-3** with *n*-butyllithium, followed by the addition of deuterium oxide. NMR showed over 95% incorporation of deuterium at the 2-position. It did not seem to be practical to make these catalysts through this approach.

2.2.2. Making an Iodo-substituted Imidazole through Halogen Exchange

2-Bromo-4,5-dicyanoimidazole is easily prepared.[181] We therefore tried to transform it to 2-iodo-4,5-dicyanoimidazole through the Finkelstein reaction. Refluxing 2-bromo-4,5-dicyanoimidazole and excess potassium iodide in acetone for three days

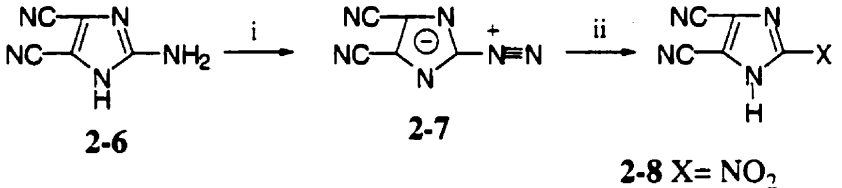
failed to produce any new product. We then applied a copper(I) iodide-assisted halogen exchange[182,183] reaction by treating **2-4** with potassium iodide and copper(I) iodide in hexamethylphosphoric triamide (HMPA) at 120 °C. The desired iodo derivative **2-5** was obtained this time (Scheme 2.3).



2-4 **2-5**

Scheme 2.3: Making 2-iodo-4,5-dicyanoimidazole

2.2.3. Making Various Catalysts through the Diazo Compound



2-6 **2-7**

2-8 X= NO_2

2-9 X=

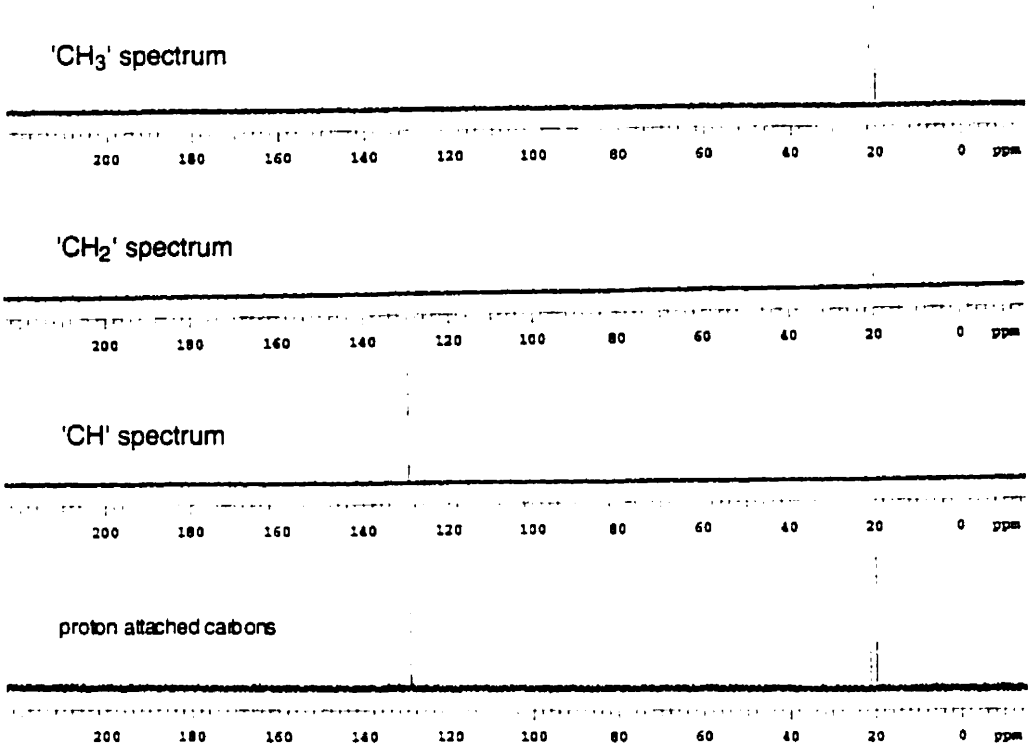
i. $NaNO_2$, HCl; ii. $NaNO_2$ or mesitylene

2-10 X=

Scheme 2.4: Making catalysts through the diazo compound

---

[181] Apen, P.G.; Rasmussen, P.G. *Heterocycles* **1989**, 29, 1325
[182] Suzuki, H.; Aihara, M.; Yamamoto, H.; Takamoto, Y.; Ogawa, T. *Synthesis* **1988**, 236
[183] Suzuki, H.; Kondo, A.; Ogawa, T. *Chem. Lett.* **1984**, 411



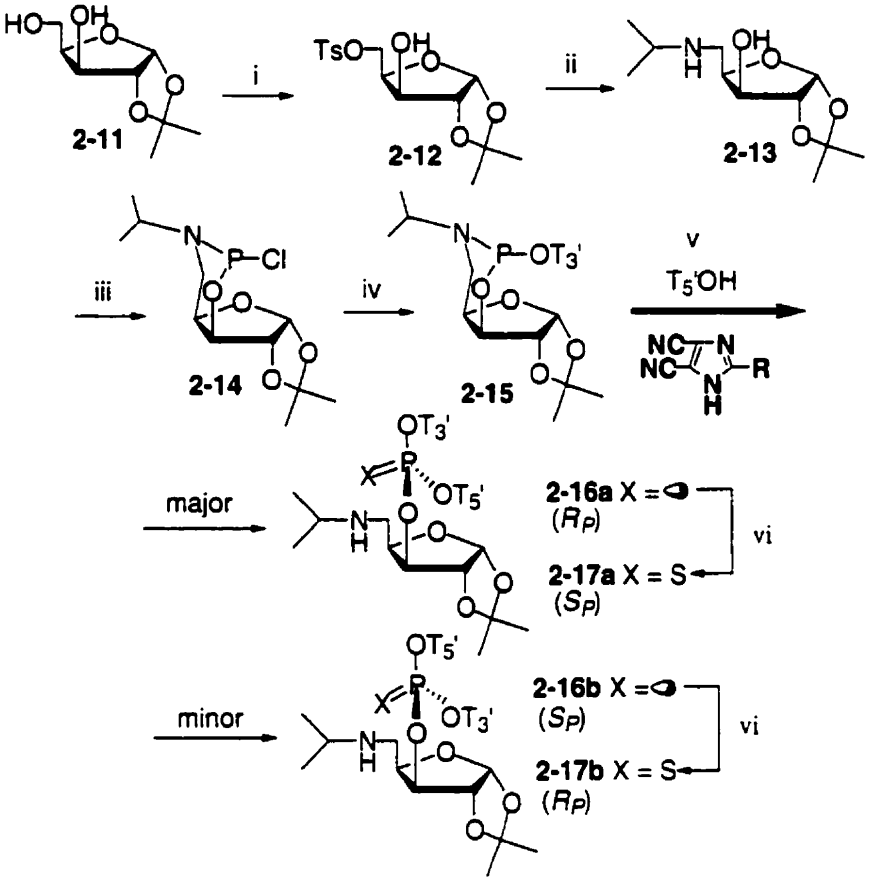
Scheme 2.5: DEPT spectra of compound **2-10**

Diazo dicyanoimidazole can be easily prepared.[184] When heated at approximately 80 °C in solution, it eliminates nitrogen to form a highly reactive intermediate that will result in the insertion of a variety of substituents at the 2-position. As shown in Scheme 2.4, 2-amino-4,5-dicyanoimidazole **2-6** was diazotized with sodium nitrite in aqueous hydrochloric acid. White 2-diazo-4,5-dicyanoimidazole **2-7** precipitated quantitatively. Diazoimidazole **2-7** has been reported[184] to be shock sensitive, and in order to avoid an explosion, wet **2-7** was transferred carefully and dried under vacuum. Reaction of **2-7** with another equivalent of sodium nitrite yielded 2-nitro-4,5-dicyanoimidazole **2-8**. Refluxing **2-7** in mesitylene gave the product of the insertion. There was no precedent in

[184] Sherooard, W. A.; Webster, O. W. *J. Am. Chem. Soc.* **1973**, 2695

the literature concerning the sites of the insertion, i. e. whether the insertion would happen at the methyl position to give a derivative of type **2-9** or on the aromatic ring directly to yield a derivative of type **2-10**. DEPT spectra of the product are shown in Scheme 2.5. There are two "CH_3" signals in ^{13}C NMR, which clearly indicates the insertion occurred at the benzene ring to give **2-10** as the product.

2.3. Test of the Catalysts in the Coupling Reaction



i. TsCl, pyridine, 0 °C; ii. *i*-$PrNH_2$, 55 °C, overnight; iii. PCl_3, Et_3N, CH_2Cl_2, 0 °C to 40 °C; iv. T_3OH, Et_3N, CH_2Cl_2, 0 °C to 50 °C; v. R=I, Br, or mesitylene; vi. Beaucage's reagent

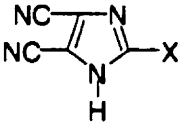
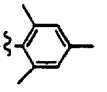
Scheme 2.6: Test of the catalysts in the coupling reaction

With the more bulky catalysts 2-iodo, 2-nitro and 2-mesityl-4,5-dicyanoimidazole **2-5**, **2-8**, **2-10** in hand, we then synthesized the phosphoramidites to find out the stereoselectivities of different catalysts in the coupling reactions.

The phosphite triester was synthesized as illustrated in Scheme 2.6. 1,2-Di-O-isopropylidene-D-xylofuranose **2-11** was tosylated selectively at the 5-position to yield **2-12**. Displacement of tosylate by isopropylamine afforded γ-amino alcohol **2-13**. After treatment with phosphorus trichloride in the presence of triethylamine, chlorophosphoramidite **2-14** was obtained. Upon heating at 40 °C to equilibrate, a single diastereomer of **2-14** having a single ^{31}P NMR resonance at around 150 ppm was obtained. Phosphorochloridite **2-14** was transformed into phosphoramidite **2-15** by reaction with T_3·OH in the presence of triethylamine. Again, a mixture of diastereomers of **2-15** obtained initially could be equilibrated by heating at 50 °C to afford a single diastereomer with a single ^{31}P NMR resonance at 130 ppm. The phosphoramidite was purified by flash chromatography. For the coupling reactions, phosphoramidite **2-15**, 3'-O-TBDMS thymidine (T_5·OH) and different catalysts, i.e. dicyanoimidazoles **2-4**, **2-5**, **2-8** or **2-10** were dried under vacuum overnight. Acetonitrile was syringed into the NMR tubes. The diastereomical ratios of two isomers were evaluated by ^{31}P NMR. Major and minor diastereomers were observed at 145 ppm and 144 ppm. Their configurations were assigned as R_P and S_P, respectively, by comparison with literature data.[185] Further sulfurization gave the corresponding phosphorothioates. Although more hindered catalysts were used, the selectivity did not improve. When iodo and mesityleneimidazoles **2-5**, **2-10** were used, the reactions went more slowly, presumably due to the their lower acidities. When nitroimidazole **2-8** was used, a major resonance at around 12 ppm was

observed. This is probably due to hydrolysis to an H-phosphonate. No work was done to establish the structure of this compound. Table 2.1 summarized these results.

Table 2.1: Selectivities of different catalysts in the coupling reactions

	X			
	Br	I		NO_2
2-16b : 2-16a	1: 6	1: 6	1: 5	Hydrolyzed

The coupling mechanism and the catalyst selectivity will be discussed in Section 3.5.

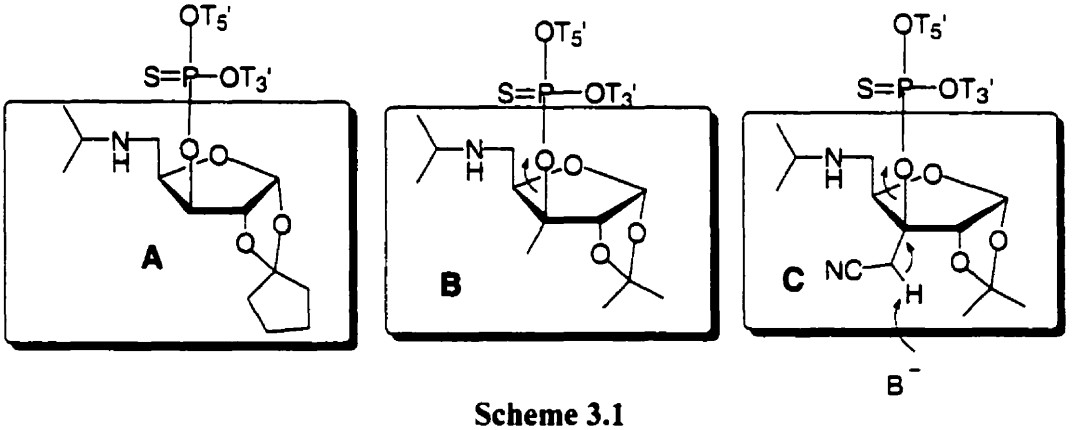
In conclusion, different catalysts **2-4**, **2-5**, **2-8** and **2-10** were synthesized. When they were applied to the coupling reactions to yield phosphite triesters, similar diastereoselectivities were observed except for the nitroimidazole **2-8** which probably formed a hydrolysis product.

[185] Jin, Y.; Just, G. *J. Org. Chem.* **1998**, 11, 3647

Chapter 3. Removable D-Xylose Derived Chiral Auxiliary as a Precursor for the Stereoselective Synthesis of Phosphorothioates

3.1. Easily Removable Chiral Auxiliaries

In our research group, 1,2-di-O-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose[171] was synthesized and used for the stereoselective synthesis of phosphorothioates (**A**). However, the removal of the chiral auxiliary at the end of the synthesis was only achieved by treatment with 70% aqueous trifluoroacetic acid. This condition seems to be too harsh if bases other than thymidine are used. We wanted to modify the chiral auxiliary so that it can be easily removed under mild conditions. Chiral auxiliaries type **B** or **C** (in the boxes) seemed to be good candidates. A S_N1 type displacement or β-elimination should allow the easy removal of the chiral auxiliaries.



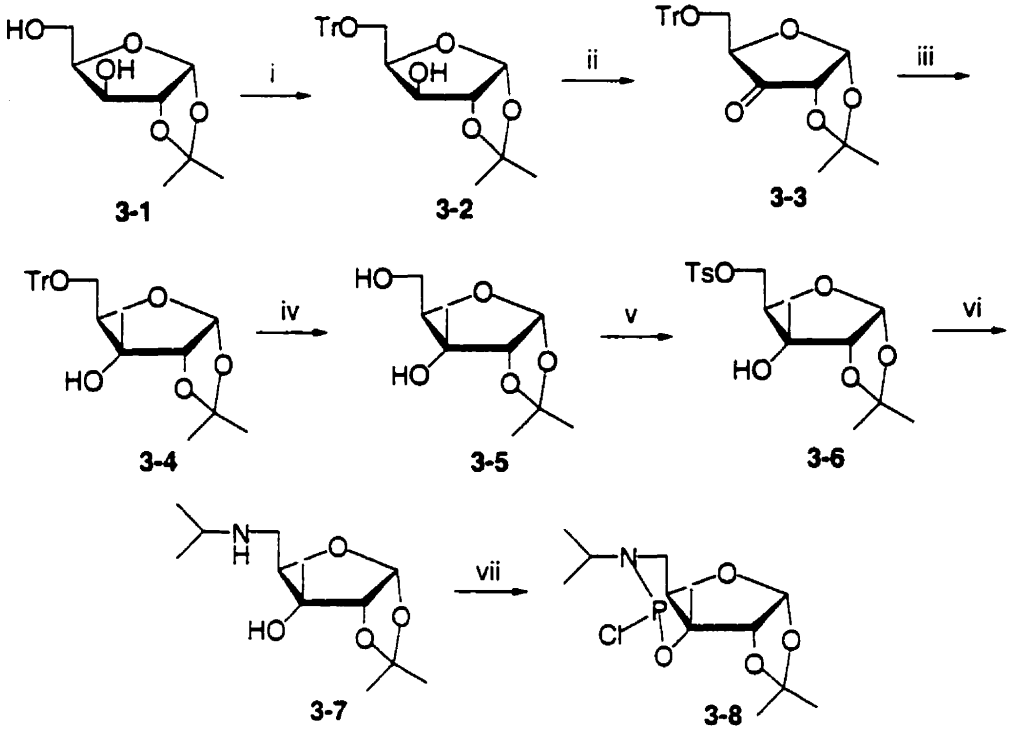
Scheme 3.1

3.2. Synthesis of a Chiral Auxiliary with a *trans*-γ-aminoalcohol structure

To construct chiral auxiliary type **B**, 1,2-di-O-isopropylidene-D-xylofuranose **3-1** was used as a readily available starting material. It is well known that the dioxolane ring

connected at position 1 and 2 of the furanose ring exerts enough steric hindrance so that nucleophiles would approach virtually exclusively from the face opposite to the dioxolane ring. Therefore, a *trans*-diol can be easily made by reaction of a Grignard reagent with the appropriate 3- ketone, and the *cis*-diol can not be prepared by this direct route because of the steric hindrance.

The synthesis of **3-7** is outlined in Scheme 3.2. Commercially available **3-1** was regioselectively protected at the 5-position. The 5-O-trityl protected **3-2** was then oxidized to ketone **3-3**, which was transformed into alcohol **3-4** by reaction with methyl magnesium bromide. *Trans*-diol **3-5** was obtained after the removal of the trityl group. Selective tosylation followed by the displacement with isopropylamine afforded the *trans*-aminoalcohol **3-7**.



i. TrCl, Et_3N, CH_2Cl_2; ii. DMSO, Ac_2O, RT; iii. CH_3MgBr, THF, 0 °C to RT; iv. AcOH, MeOH, reflux; v. TsCl, pyridine, 0 °C; vi. *i*-$PrNH_2$, 55 °C; vii. PCl_3, Et_3N, CH_2Cl_2

Scheme 3.2: *Trans*-aminoalcohol **3-7** as a chiral auxiliary

We then proceeded to see if cyclic phosphoramidite could be formed efficiently. *Trans*-aminoalcohol **3-7** in dichloromethane containing triethylamine was added slowly to a solution of phosphorus trichloride in dichloromethane in a scrupulously dried NMR tube, the NMR tube was then sealed. ^{31}P NMR revealed several peaks ranging from 10 ppm to 220 ppm after the addition. However, the equilibration of the mixture failed to produce any major products. As indicated by ^{31}P NMR, a wide range of resonances was observed even after heating at 50 °C for two days. We concluded from this experiment that it is necessary to have hydroxy and amino group *cis* to each other in the chiral auxiliary in order to have an efficient six-membered ring formation.

3.3. Chiral Auxiliary with a tertiary Alcohol *cis* to the Amino Group

Our next attempt was to convert the *trans*-diol **3-5** into a *cis* diol (Scheme 3.3). It has been shown[186,187] that 17β-methyl-17α-hydroxy steroid (**a**) could be produced from its 17β-hydroxyl-17α-methyl epimer (**b**) by means of sulfonation, followed by a stereospecific hydrolysis. We therefore reacted *trans*-diol **3-5** with chlorosulfonic acid and pyridine to produce sulfate **3-9**. It was then left in a pH 7 buffer solution overnight. Several products were obtained, probably due to an elimination reaction. No desired product corresponding to **3-11** could be isolated. Scheme 3.3 depicts the attempted inversion.

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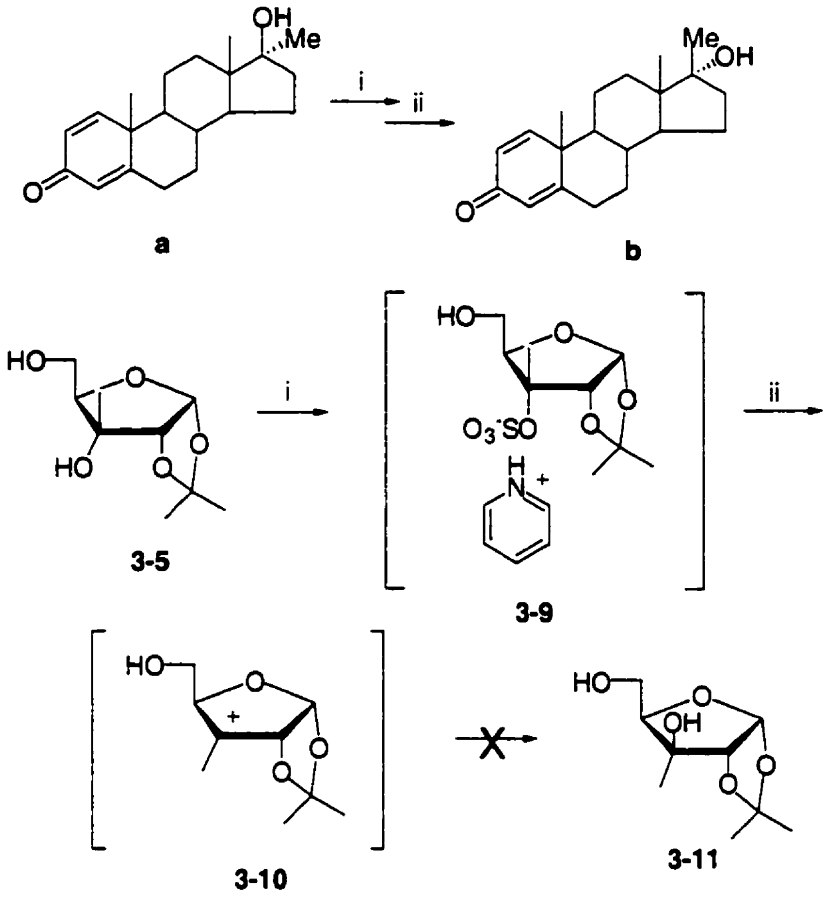
[186] Edlund, P. O.; Bowers, L.; Henion, J. H. *J. Chromatogr.* **1989**, 487, 341
[187] Bi, H.; Masse, R. and Just, G. *Steroids* **1992**, 57, 306

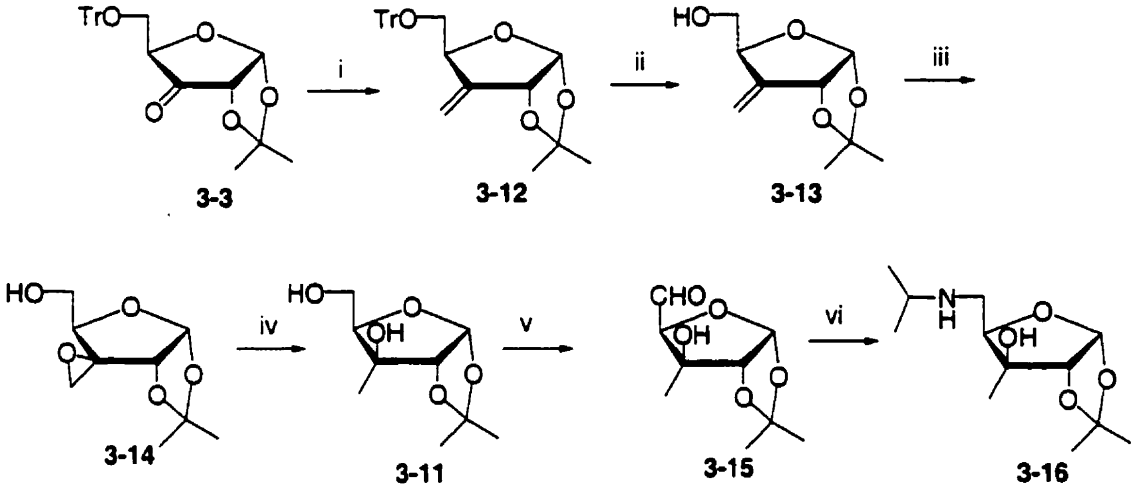


i. $ClSO_3H$, Pyridine, 0 °C to RT; ii. PH 7 buffer

Scheme 3.3: Reversion of *trans* diol **3-5**

We then synthesized chiral auxiliary **3-16** as illustrated in scheme 3.4. Ketone **3-3** was transformed into alkene **3-12** via a Wittig reaction. Reaction of **3-12** with 3-chloroperoxybenzoic acid (MCPBA) for one day failed to produce any desired epoxide. The trityl group was then removed to furnish 5-hydroxy alkene **3-13**, which was treated with MCPBA to yield the desired epoxide **3-14**. Since the epoxide **3-14** and the alkene **3-13** had almost identical R_f values, the progress of this epoxidation reaction had to be followed by ^1H NMR instead of TLC. Lithium aluminum hydride reduction opened the epoxide to afford diol **3-11**, whose *cis*-configuration was confirmed by NMR studies. 2D-

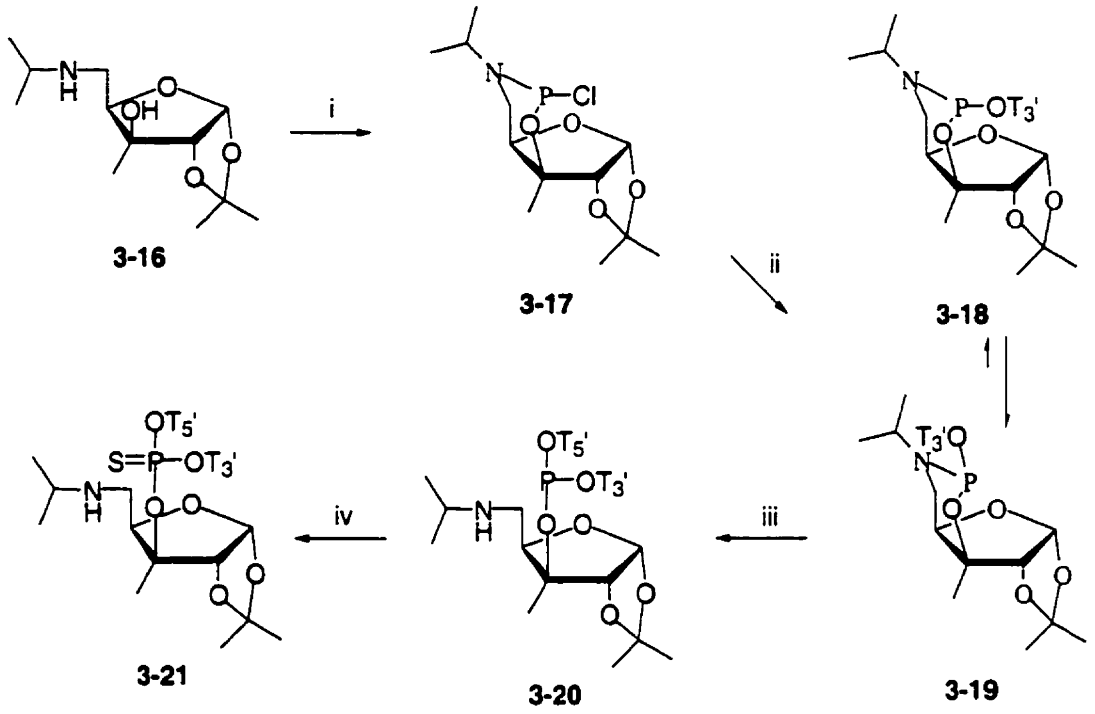
NOSEY spectra were run on both diols **3-5** and **3-11** to establish the stereochemistry. In the spectrum of the former one, there were strong cross peaks between 3-methyl group and the 1-, the 2- and 5- protons, which clearly indicated *trans*-diol configuration. For the latter one, cross peaks were observed between the 3-methyl group and the 4-proton, which suggested that the methyl group pointed downwards. Having proved the stereochemistry, *cis*-diol **3-11** was oxidized to aldehyde **3-15** using Dess-Martin periodinane,[188,189,190] followed by reductive amination employing sodium triacetoxyborohydride[191] and isopropylamine to complete the synthesis of *cis*-γ-aminoalcohol **3-16**.



i. CH_3PPh_3Br, NaH, THF, 0 °C to RT; ii. AcOH, MeOH, Reflux; iii. MCPBA, CH_2Cl_2, RT; iv. $LiAlH_4$, THF; v. Dess-Martin reagent, CH_2Cl_2; vi. $NaB(OAc)_3H$, *i*-$PrNH_2$, DCE, RT

Scheme 3.4: Synthesis of *cis*-γ-aminoalcohol

[188] Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, 48, 4155
[189] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, 113, 7277
[190] Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, 58 2899
[191] Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoft, C. A. and Shah, R. O. *J. Org. Chem.* **1996**, 61, 3849



i. PCl_3, Et_3N, CH_2Cl_2, 0 °C to RT; ii. T_3OH, Et_3N, CH_2Cl_2, 0 °C to RT; iii. T_5-OH, 2-bromo-4,5-dicyanoimidazole, $CDCl_3$, 0 °C; iv. Beaucage's reagent

Scheme 3.5: Synthesis of phosphoramidites and phosphorothioates

The synthesis of diastereomerically pure phosphoramidite **3-19** is illustrated in Scheme 3.5. γ-Aminoalcohol **3-16** and triethylamine in dichloromethane were added slowly to phosphorus trichloride at 0 °C. The mixture was then allowed to warm up to room temperature. ^{31}P NMR showed a single resonance at 149.6 ppm immediately, which is typical for this type of phosphorochloridite. Without isolation, the reaction mixture was cooled down to 0 °C and treated with T_3-OH in dry dichloromethane. After warming up to room temperature, ^{31}P NMR showed the formation of two new resonances at 131.6 ppm and 132.4 ppm in a ratio of 14 to 1, which were assigned to **3-19** and **3-18** (structural assignments will be discussed in Section 3-5).

Purification by flash chromatography furnished the cyclic phosphoramidite **3-19** as a single diastereomer. Phosphoramidite **3-19**, T_5·OH and 2-bromo-4,5-dicyanoimidazole were dried under vacuum for 15 hours. Freshly distilled deutero-chloroform was then added slowly at 0 °C and the mixture was kept at this temperature for 4 hours. ^{31}P NMR revealed two resonances at 139.6 ppm and 139.3 ppm, in a ratio of 8 to 1, which were assigned to the two isomers of **3-20**. The mixture was treated with Beaucage's reagent, and resulted in the formation of products with resonances at 60.7 ppm and 60 ppm in a ratio of 1 to 8. For the similar systems,[171,185,192] typical phosphorothioates of similar types would have ^{31}P NMR resonances at about 68 ppm, and the resonances of the deprotected dithymidine phosporothioate dimers should appear at 55-57 ppm. A 60 ppm resonance is close to the latter. The product was then purified and characterized as **3-21** or a closely related isomer. ^{1}H NMR indicated the presence of the sugar chiral auxiliary and the mass spectrum also proved the attachment of the sugar moiety.

We then tried to remove the chiral auxiliary under mild conditions. Dimer **3-21** was first treated with concentrated ammonia at 55 °C for 16 hours. ^{31}P NMR did not show any changes after the reaction. We proceeded to separate and characterize the products. Much to our surprise, the chiral auxiliary moiety in **3-21** was still attached. We reasoned that acidic conditions might facilitate the removal of the chiral auxiliary better via a S_N1 mechanism. Therefore **3-21** was reacted with 3% aqueous trifluoroacetic acid (TFA) solution at room temperature. The product of the reaction appeared to be the same, as indicated by ^{31}P NMR. ^{1}H NMR and mass spectrum further showed that no change occurred to **3-21**.

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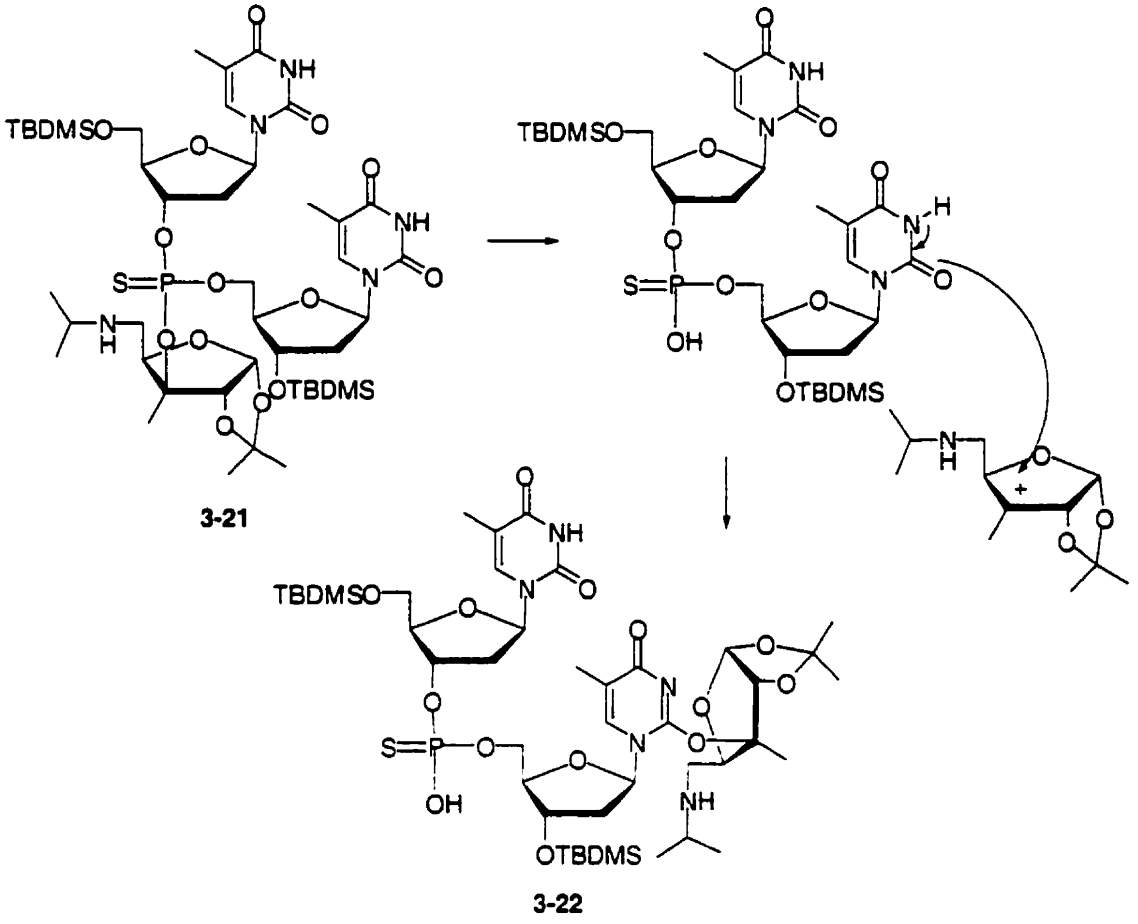
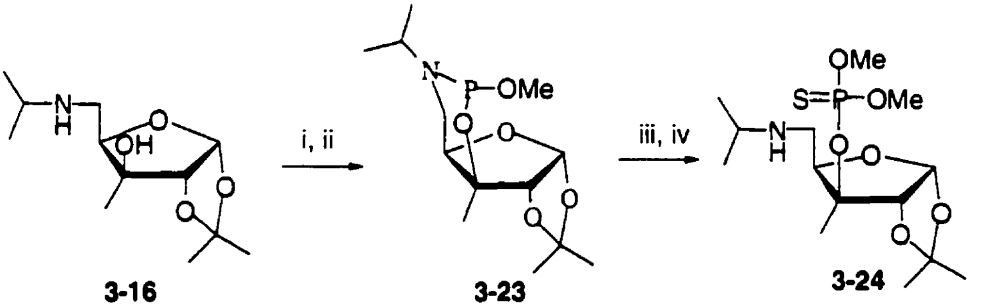
[192] Chapter 2 of this thesis.

We suspected there might be some other structural variations to **3-21**. For instance, **3-22** could be a reasonable alternative structure to **3-21**. Its possible formation is shown in Scheme 3.6. Upon the formation of **3-21** in the coupling reaction, it might spontaneously break down into a chiral auxiliary carbocation part and phosphorothioate. The reaction between thymidine exocyclic base and carbocation could result in the formation of species like **3-22**. A structure in which the sulfur of the thiophosphate is attached to the tertiary carbon atom can be excluded because they have a ^{31}P NMR resonance at around 30 ppm (see Chapter 4, Section 4.3).



Scheme 3.6: Possible rearrangement of **3-21**

To suppress such possible side reactions, the coupling reaction was repeated using excess anisole as a carbocation scavenger. The same results were obtained. This seemed to make such a rearrangement unlikely. To further prove this, we carried out reactions using a simpler alcohol such as methanol, as depicted in Scheme 3.7. The chiral auxiliary was first reacted with phosphorus trichloride, then with methanol to form cyclic phosphoramidite **3-23**, which was coupled with another equivalence of methanol, followed by sulfurization to afford phosphorothioate **3-24**. The clear presence of the chiral auxiliary moiety in **3-24** rules out the possibility of spontaneous formation of the carbocation in the coupling reaction.



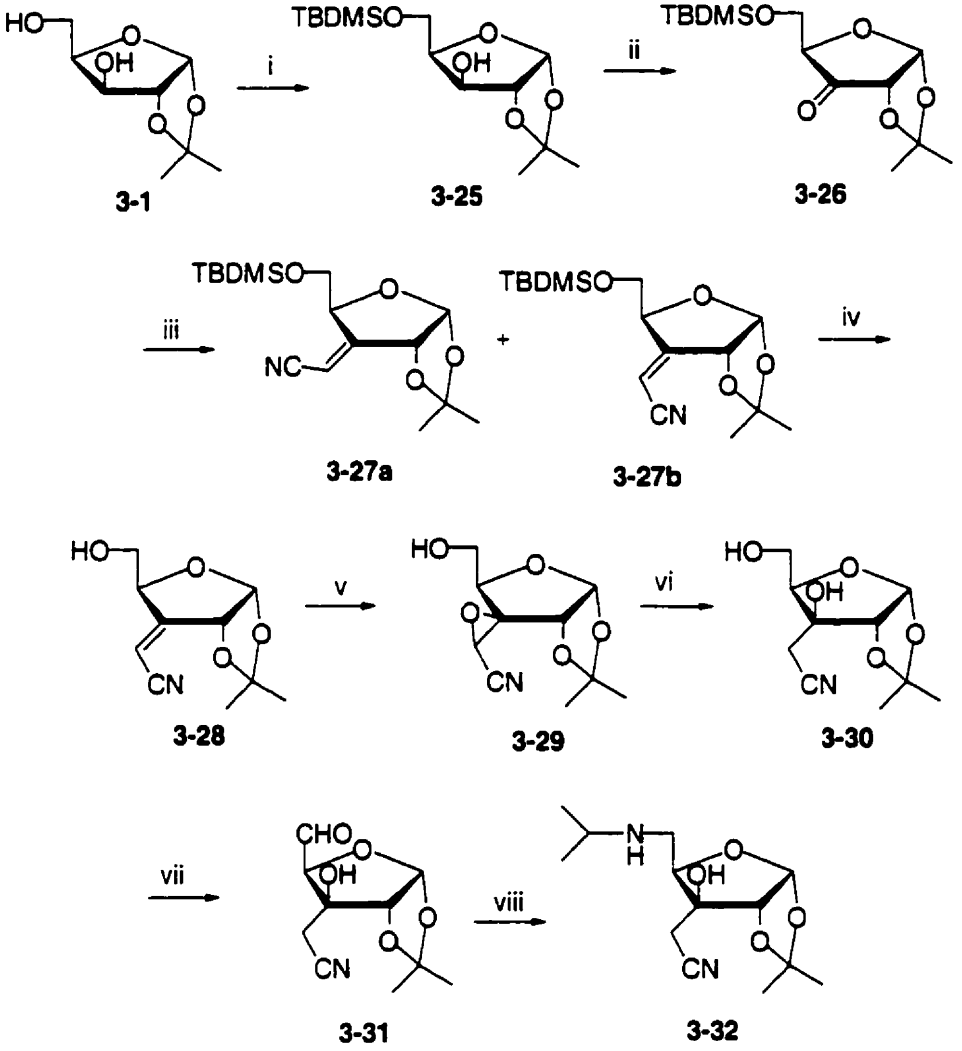
i. PCl_3, Et_3N, CH_2Cl_2, 0 °C to RT; ii. MeOH, Et_3N, CH_2Cl_2; iii. MeOH, 2-bromo-4,5-dicyanoimidazole, CH_2Cl_2; iv. Beaucage's reagent

Scheme 3.7: Synthesis of dimethylphosphorothioate **3-24**

To conclude this work, chiral auxiliary **3-16** could be used as a precursor for the stereoselective synthesis of phosphorothioate triesters, but its removal at the end of the synthesis could not be realized.

3.4. Chiral Auxiliary with a β-cyano Group

Our next effort was to synthesize chiral auxiliary type **C** (Scheme 3.1). Such a chiral auxiliary (**3-32**) should be very easily removed by β-elimination.



i. TBDMSCl, imidazole, DMF; ii. CrO_3, Pyridine, Ac_2O, RT; iii. $CNCH_2P(O)(OEt)_2$, NaH, THF, 0 °C to RT; iv. TBAF, 0 °C; v. Triton-B, TBHP, THF; vi. $LiBH_4$, diglyme; vii. $(COCl)_2$, DMSO, CH_2Cl_2; viii. *i*-$PrNH_2$, $Na(OAc)_3BH$, DCE, RT.

Scheme 3.8: Chiral auxiliary with β-cyano group

The synthesis started with commercially available D-xylose derivative **3-1**, which was first protected regioselectively at the 5-position as its silyl ether **3-25**, and then oxidized to ketone **3-26**. Wittig reaction of **3-26** with diethyl cyanomethylphosphonate then afforded alkenes **3-27a** and **3-27b** in a ratio of 1 to 5, which were separated by flash chromatography. The structural determination of the two isomers was based on ^{1}H NMR. In *E*-isomer **3-27a**, the chemical shift of the 4-proton shifted more to downfield due to the closeness of the cyano group, while in the *Z*-isomer **3-27b**, the neighboring cyano group made the 2-proton move downfield. The major isomer **3-27b** was deprotected to give **3-28**, which was treated with *N*-benzyltrimethylammonium hydroxide (triton-B) and *tert*-butyl hydroperoxide (TBHP) to furnish epoxide **3-29**. Samarium diiodide[193] mediated reduction of the α,β-epoxy ketone was unsuccessful, and only the starting material was recovered. The selective reduction of the epoxide was achieved when **3-29** was reacted with lithium borohydride in diglyme. Swern oxidation of diol **3-30** followed by reductive amination afforded the desired γ-aminoalcohol chiral auxiliary **3-32** (Scheme 3.8).

The synthesis of phosphoramidites and phosphorothioates are depicted in Scheme 3.9. γ-Aminoalcohol **3-32** was treated with phosphorus trichloride in the presence of triethylamine. ^{31}P NMR indicated that a major peak at 147.6 ppm was formed immediately, which was assigned to **3-33**. Without isolation, the reaction mixture was treated with T_3·OH in dichloromethane. The ^{31}P NMR resonance at 147.6 ppm shifted to 133.1 ppm, indicating the formation of phosphoramidite. Purification by flash chromatography furnished **3-34** as a single diastereomer.

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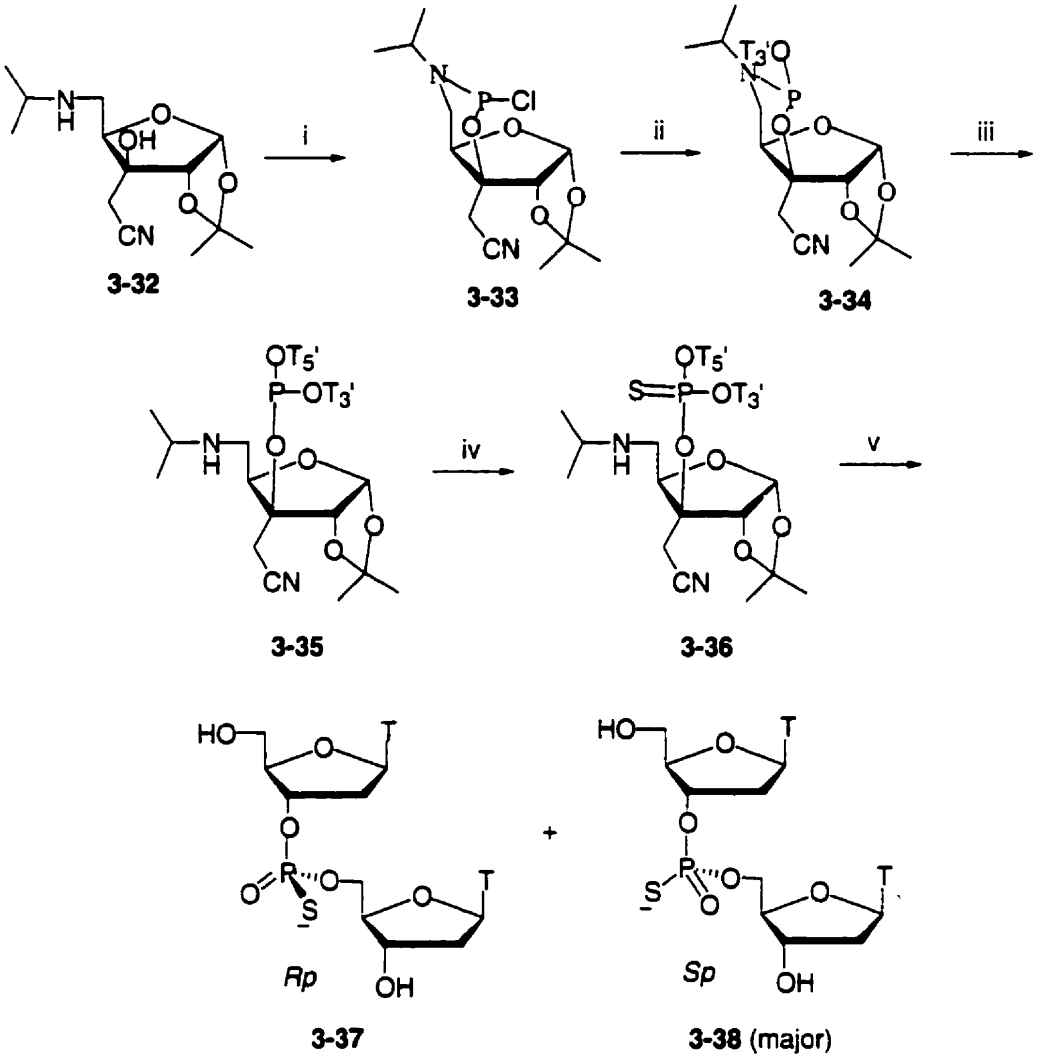
[193] Molander, G. A. and Hahn, Gregory *J. Org. Chem.* **1986**, 51, 2596



i. PCl_3, Et_3N, CH_2Cl_2, 0 °C to RT; ii. T_3OH, Et_3N, CH_2Cl_2, 0 °C to RT; iii. T_5'OH, 2-bromo-4,5-dicyanoimidazole, $CDCl_3$, 0 °C; iv. Beaucage's reagent; v. conc. ammonia, RT, then TBAF

Scheme 3.9: The synthesis of phosphoramidite and phosphorothioates from **3-32**

The coupling reaction between phosphoramidite **3-34** and T_5'OH was then carried out, using 2-bromo-4,5-dicyanoimidazole as the catalyst. Phosphite triesters **3-35** were obtained, as established by ^{31}P NMR (138.5 ppm and 137.5 ppm in a ratio of 1 to 6). Without purification or equilibration, phosphite triesters were sulfurized to give

phosphorothioates **3-36** with a ^{31}P NMR at 59.05 ppm and 59.13 ppm in the same ratio. After treatment with concentrated ammonia solution at room temperature for 5 minutes, to our delight, the peaks originally at 59 ppm shifted to 57.3 ppm and 56.8 ppm, in a ratio of 1 to 6. The silyl protecting groups were removed to give dithymidine phosphorothioates **3-37** (56.8 ppm) and **3-38** (56.6 ppm) in a ratio of 1 to 6. The configuration of **3-37** and **3-38** were assigned as *Rp* and *Sp*, by comparison of ^{31}P NMR and HPLC data.

In summary, the use of D-xylose derived chiral auxiliary **3-32** allows the stereoselective synthesis of *Sp* dithymidine phosphorothioate **3-38**. The chiral auxiliary was easily removed by rapid reaction with concentrated ammonia at room temperature.

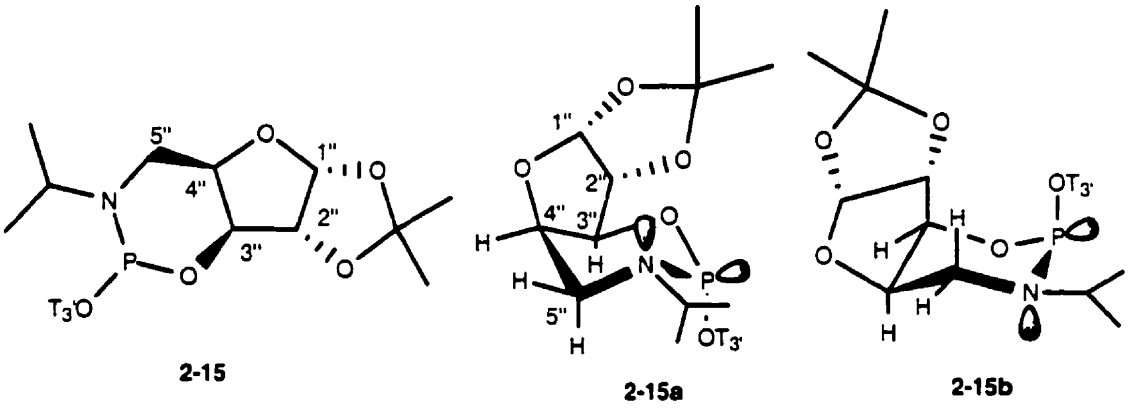
3.5. The Mechanism of the Coupling Reaction

The mechanism of the coupling reaction 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 will attack as a nucleophile giving rise to the formation of an azolide intermediate[194]. In the first case, after protonation of phosphoramidite, T_5·OH reacts to give a phosphite triester with inversion of configuration. The second process involves first displacement of the protonated amino part of phosphoramidite by azole, followed by displacement by an alcohol which gives the desired phosphite triester with retention of configuration.

3.5.1. The Structural Assignments for the Phosphoramidites

[194] Berner, S.; Muhlegger, K.; Seliger, H *Nucleic Acids Res.* **1989**, 17, 853

The structural assignment for the phosphoramidite **2-15** was made according to the literature.[195,196,197] It was shown by Bentrude and co-workers that in bicylcic six-membered ring phosphite, the 1,3,2-dioxaphosphorinane ring preferentially adopted a chair conformation with the exocyclic OR group attached to the phosphorus in an axial position. Therefore, the thermodynamically more stable isomer of phosphoramidite **2-15** would adopt a chair form with thymidine attached axially at the exocyclic position. Between the two possible isomers **2-15a** and **2-15b**, the former one should be strongly favored due to the far smaller 1,3-diaxial interaction (Scheme 3.10).



Scheme 3.10: Two possible isomers of **2-15**

The axial position of OT_3· was based on ^{13}C NMR data: the large coupling constant (19.2 Hz) between C-3' of thymidine and phosphorus, a small coupling constant (3.7 Hz) for C-O-P of the oxazaphosphorinane carbon and a small coupling constant (1.8 Hz) for C-4'' of the chiral auxiliary and the phosphorus.

Table 3.1 lists the selected carbon and phosphorus coupling constants for phosphoramidites **2-15**, **3-19** and **3-34**.

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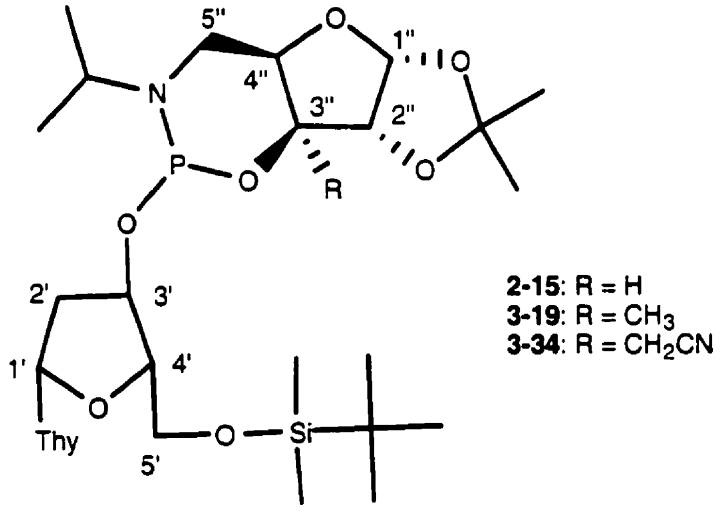
[195] Huang, Y.; Yu, J.; Bentrude, W. G. *J. Org. Chem.* **1995**, 60, 4767

Table 3.1 Selected carbon-phosphorus coupling constants of phosphoramidites

Compounds	$J_{C3''-P}$	$J_{C3''-P}$	$J_{C5''-P}$	J_{CHN-P}
2-15	19.2 Hz	3.7 Hz	2.8 Hz	44.0 Hz
3-19	21.1 Hz	8.2 Hz	4.5 Hz	37.6 Hz
3-34	23.8 Hz	8.3 Hz	3.6 Hz	38.5 Hz



The C-3'' and phosphorus ($J_{C3''-P}$) coupling constants for **3-19** and **3-34** are much larger than the one of **2-15**, which indicated a different configuration for the 3''-alkylphosphoramidites **3-19** and **3-34** as compared to **2-15**, which has a hydrogen at the C-3'' position.

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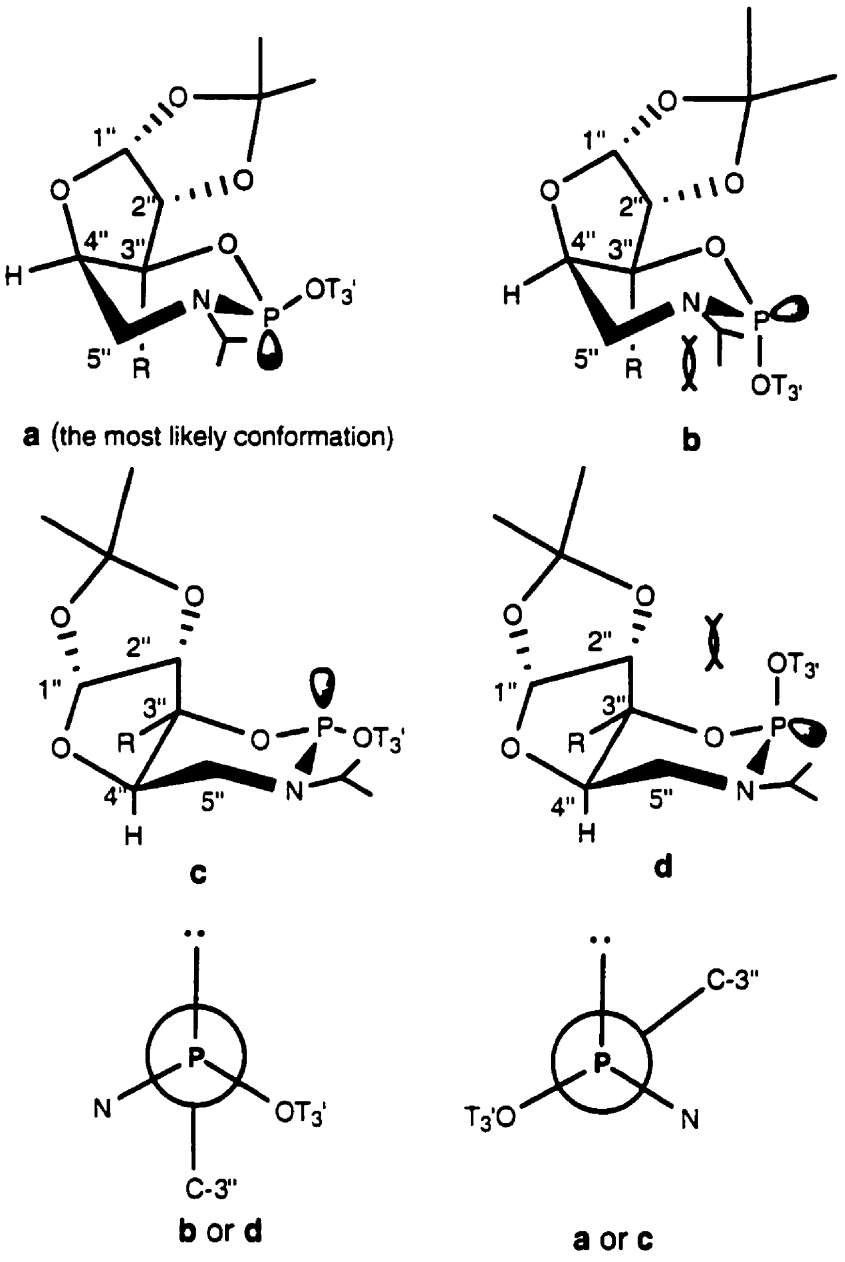
[196] Nelson, K.A.; Sopchik, A. E.; Bentrude, W. G. *J. Am. Chem. Soc.* **1983**, 105, 7752

[197] Van Heeswijk, W. A. R.; Goedhart, J. B.; Vliegenthart, J. F. G. *Carbohyd. Res.* **1977**, 58, 337



Scheme 3.11: The possible configurations for **3-19** and **3-34**

There are two possible configurations and four possible conformations for **3-19** or **3-34**, depending on whether OT_3· is axially or equatorially disposed. The two

conformations (**b** or **d**) where $OT_{3'}$ is axially disposed are unfavorable due to the strong 1,3-dipolar interactions. Such interactions would be avoided if $OT_{3'}$ adopted an equatorial or pseudoequatorial positions (**a** or **c**).

The equatorial disposition of the $OT_{3'}$ group was confirmed by C-3'' and phosphorus coupling constants. It is well documented in the literature[198,199,200] that the carbon to phosphorus coupling constants ($J_{C\text{-}P}$) in trivalent phosphorus derivatives are highly dependent upon the relative spatial orientation of the lone pair on phosphorus and the carbon atom. Haemer et al. reported[201,202] the amplitudes of the $J_{C\text{-}P}$ coupling constants in a series of rigid dioxaphosphorinanes depend on the dihedral angle between the phosphorus lone pair and the O-C bond. A dihedral angle close to 0° will induce a large coupling constant, whereas a dihedral angle close to 180° will correspond to a small coupling constant. In the Newman projection of configuration **b** or **d**, the dihedral angle between the phosphorus lone pair and the O-C-3'' bond is close to 180°, and a smaller coupling constant (3.7 Hz) is expected for these two conformers. When the dihedral angle is 60° as illustrated for **a** or **c**, larger C-3'' and phosphorus coupling constants (8.2 Hz) are observed.

We then carried our molecular modeling to differentiate between configurations **a** and **c**. Calculations were done by applying the MacroModel program (version 5.5). Monte-Carlo search was carried out to find out the global minima for **a** or **c**. The calculations show that configuration **a** is about 3.5 kcal/mole more stable than **c**. Therefore, the most likely configuration for **3-19** or **3-34** is **a**.

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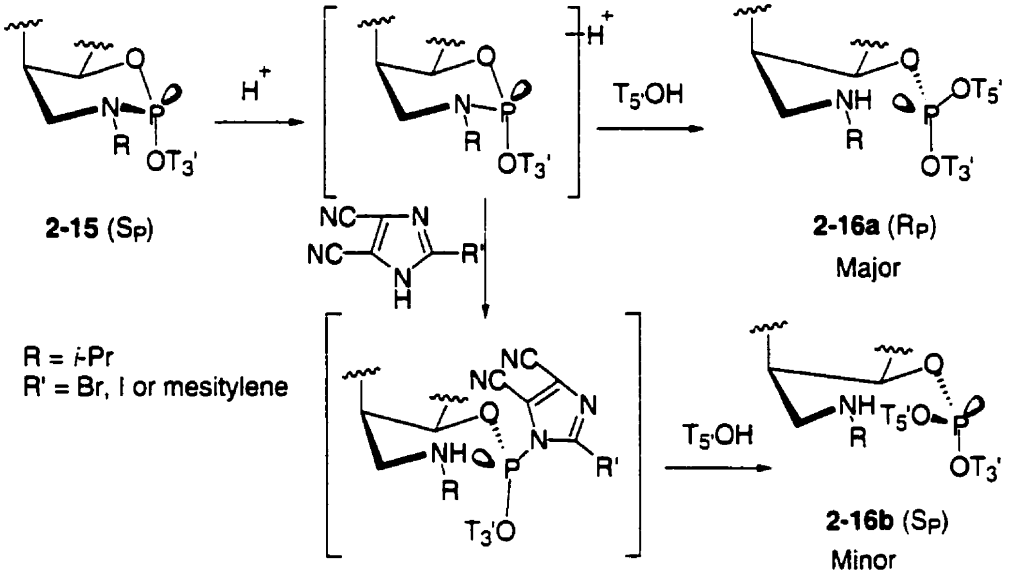
[198] White, D. W.; Bertrand, R. D.; McEwen, G. K.; Verkade, J. G. *J. Am. Chem. Soc.* **1970**, 92, 7125
[199] Breen, J. J.; Featherman, S. I.; Quin, L. D.; Stocks, R. C.; *J. Chem. Soc. Chem. Commun.* **1972**, 657
[200] Sorensen, S.; Hansen, R. S.; Jakobsen, H. J.; *J. Am. Chem. Soc.* **1972**, 94, 5900
[201] Haemers, M.; Ottinger, R.; Zimmermann, D.; Keisse, J.*Tetrahedron Lett.* **1973**, 24, 224

3.5.2. The Coupling Mechanisms

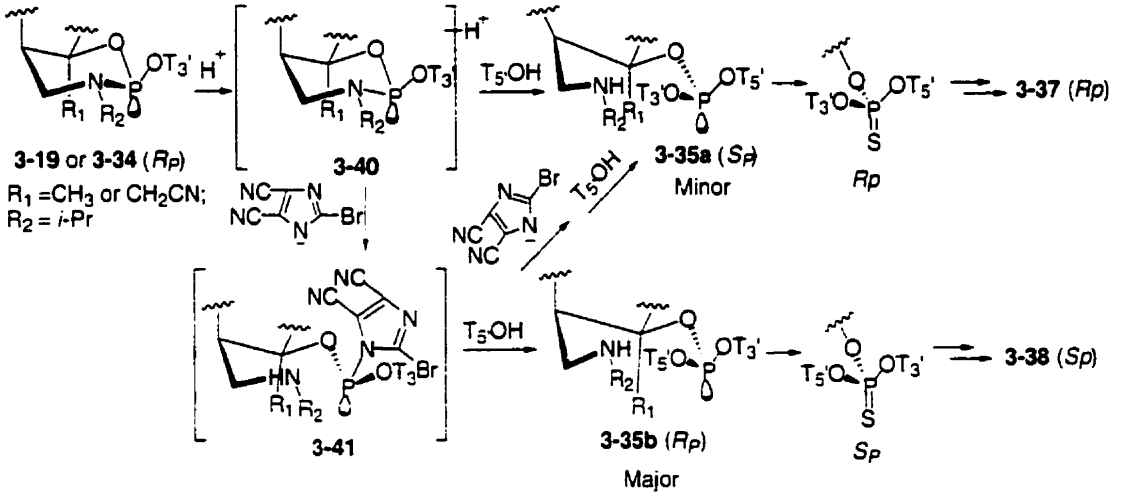
For the coupling reactions described in Section 2.3. A careful examination of the stereochemistry of **2-15** (S_P) and **2-16a** (R_P) indicated there was only one inversion during the displacement process (Scheme 3.12). The mechanism we therefore propose is that after the protonation of phosphoramidite **2-15**, T_5·OH attacks directly the phosphorus center to yield phosphite triester **2-16a** as a major diastereomer. This result is in agreement with a similar system.[185] The mechanism is further confirmed by comparing the diastereoselectivities of the coupling reactions when the different catalysts (**2-4**, **2-5**, **2-8** and **2-10**) were used. Although the catalyst sizes were different, the selectivity remained about the same. This can be understood if the sole role of the catalysts had been to act as a proton source.



Scheme 3.12: The mechanism of the coupling reaction of **2-15**

[202] Haemers, M.; Ottinger, R.; Zimmermann, D.; Keisse, J.*Tetrahedron* **1973**, 29, 3538

For the coupling reactions employing **3-19** or **3-34**, analysis of the stereochemistry of **3-34** (*Rp*) and the major isomer of dithymidine phosphorothioate **3-38** (*Sp*) indicated that the transformation involved double inversion. The following mechanism is proposed. The *Rp*-phosphoramidite **3-19** or **3-34** is first protonated by the catalyst to give **3-40**. The catalyst then displaces the protonated amine to form imidazolide intermediate **3-41**, which is displaced by thymidine to give the desired *Rp*-phosphite triester **3-35b**. Sulfurization and the deprotection then yield dithymidine phosphorothioate **3-38** (*Sp*) as the major isomer. Imidazolide **3-41** can be displaced by another molecule of the catalyst and lead to epimerization. The competing reaction of T_5·OH with **3-40** can be another source of the epimerization. The double displacement mechanism is further confirmed when a bigger catalyst, 2-mesitylene-4,5-dicyanoimidazole was used as the activator in the coupling reaction of **3-19**. The same phosphite triesters were obtained in a ratio of 15 to 1, as compared to a ratio of 6 to 1 when 2-bromo-4,5-dicyanoimidazole was used as a catalyst under the same conditions.



Scheme 3.13: The mechanism of the coupling reaction

In conclusion, our results suggested both the single protonation mechanism and the double inversion mechanism are the possible pathways for the coupling reactions.

Chapter 4. Allyl as a Protecting Group for Internucleotide Phosphates and Phosphorothioates

4.1. Substituted Allyl Groups as Protecting Groups

In the routine solid phase synthesis of oligonucleotides, the typical protecting group for the internucleotide linkage is the β-cyanoethyl group. It was first used by Letsinger and Ogilvie[123] in the "phosphite triester" approach and later introduced by Sinha et al.[203,204] as the protection of the phosphate function in the phosphoramidite approach. Despite the popularity that the β-cyanoethyl group enjoyed, there are some significant disadvantages concerning its use. For example, the cyanoethyl group is base labile because of the potential β-elimination, and the resulting acrylonitrile can at times reacts with the trivalent phosphorus species to give cyanoethylphosphonate.[177]

In the course of developing chiral auxiliaries for the solid support synthesis of oligonucleotide phosphorothioates having a high diastereomeric excess[185], we required a protecting group stable to bases, such as DBU, or fluoride, and preferably removable under neutral conditions. Allyl and substituted allyl groups seemed to be good candidates. The allyl group has been used[205,206,207,208] as an alternative to the cyanoethyl group. Palladium(0) complexes were used to deblock the allyl group. The palladium catalyzed deprotection is inconvenient and limiting for various reasons. Palladium complexes are not practical for use in the large scale synthesis. Furthermore, during the deprotection,

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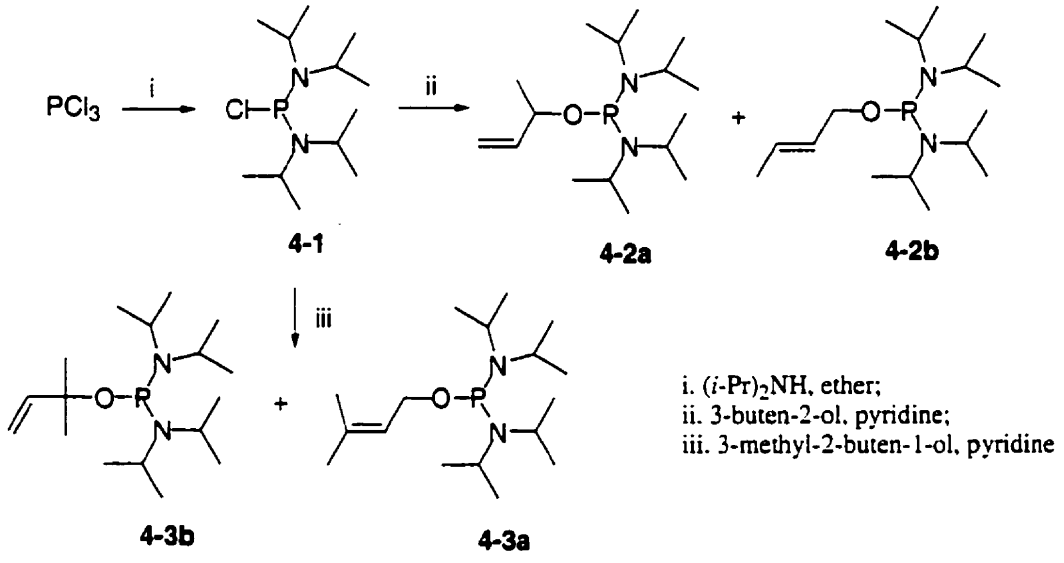
[203] Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucl. Acids Res.* **1984**, 12, 4539
[204] Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, 24, 5843
[205] Hayakawa, Y.; Wakabayashi, S.; Kato, H.; and Noyori, R. *Tetrahedron. Lett.* **1985**, 26, 6505
[206] Noyori, R.; Uchiyama, M.; Kato, H.; Wakabayashi, S. and Hayakawa, Y. *Pure & Appl. Chem.* **1990**, 62, 613

palladium catalysts are easily poisoned, especially with phosphorothioate oligonucleotides. Moreover, traces of palladium catalysts may remain in the product after the deprotection and purification procedure. This can be toxic when the product is used therapeutically. We reasoned that a S_N2 or a S_N2' displacement by nucleophiles should be able to remove the allyl protecting group at the end of a synthetic sequence. We therefore tried to synthesize dithymidine phosphates and phosphorothioates with allyl or substituted allyl protecting groups and test the feasibility of their removal by nucleophilic attack.



Scheme 4.1: Substituted allyl as protecting groups

We first attempted to synthesize substituted allyl tetraisopropyl phosphorodiamidites by reaction of chloroisopropylphosphorodiamidite with 3-buten-2-

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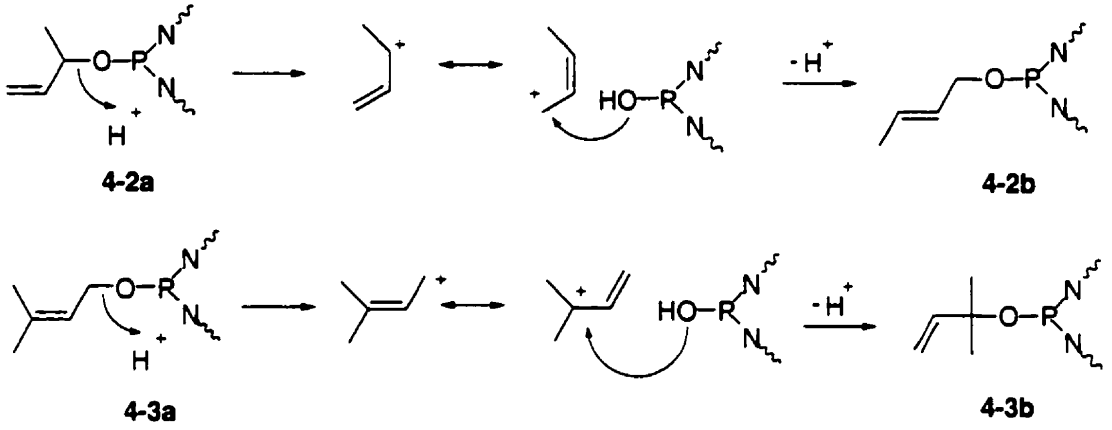
[207] Hayakawa, Y.; Wakabayashi, S.; Kato, H. and Noyori, R. *J. Am. Chem. Soc.* **1990**, 112, 1691
[208] Hayakawa, Y.; Hirose, M. and Noyori, R. *J. Org. Chem.* **1993**, 58, 5551

ol or 3-methyl-2-buten-1-ol (Scheme 4.1). The desired phosphoramidites **4-2a** or **4-3a** were obtained mixed with the rearranged products **4-2b** or **4-3b**, and could not be separated by column chromatography. The rearrangement is described in Scheme 4.2.



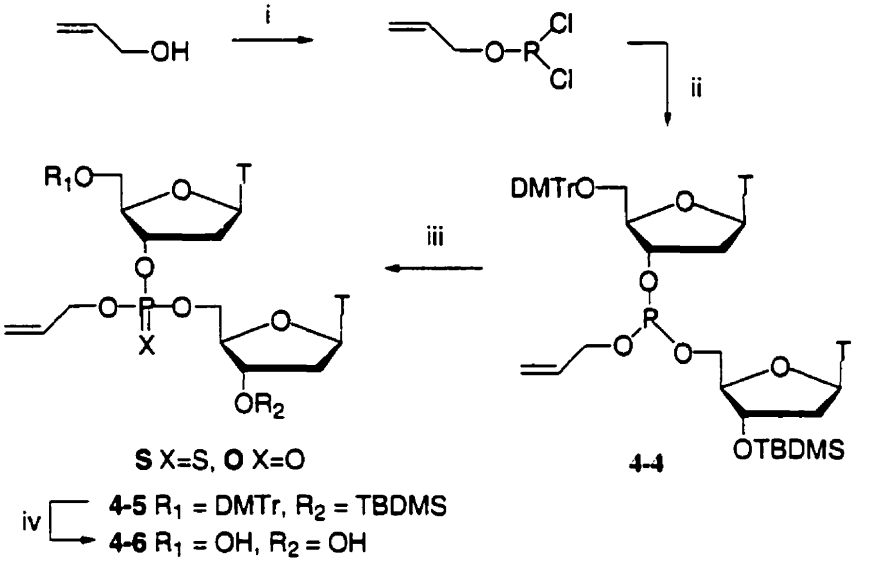
Scheme 4.2: Rearrangement of substituted allyl phosphoramidite

4.2. Syntheses of Phosphorothioate and Phosphate Dinucleotides

We then turned to the allyl protection. Allyl protected dithymidine phosphates and phosphorothioates were easily prepared using both phosphite triester[127,128] and phosphoramidite[129] approaches.

In the phosphite triester approach (Scheme 4.3), freshly distilled allyl alcohol in dry ether was added slowly over a period of one hour into a ether solution of phosphorus trichloride and pyridine at -78°C, and the mixture was stirred at -78°C for 15 minutes. Pyridine and 5'-O-DMTr-thymidine in dry THF were then added, followed by the addition of pyridine and 3'-O-TBDMS-thymidine in dry THF. Without purification,

either iodine or Beaucage's reagent was introduced to oxidize or sulfurize **4-4** to give **4-5S** or **4-5O**. The trityl and TBDMS groups were removed in the standard manners to afford **4-6S** or **4-6O**.

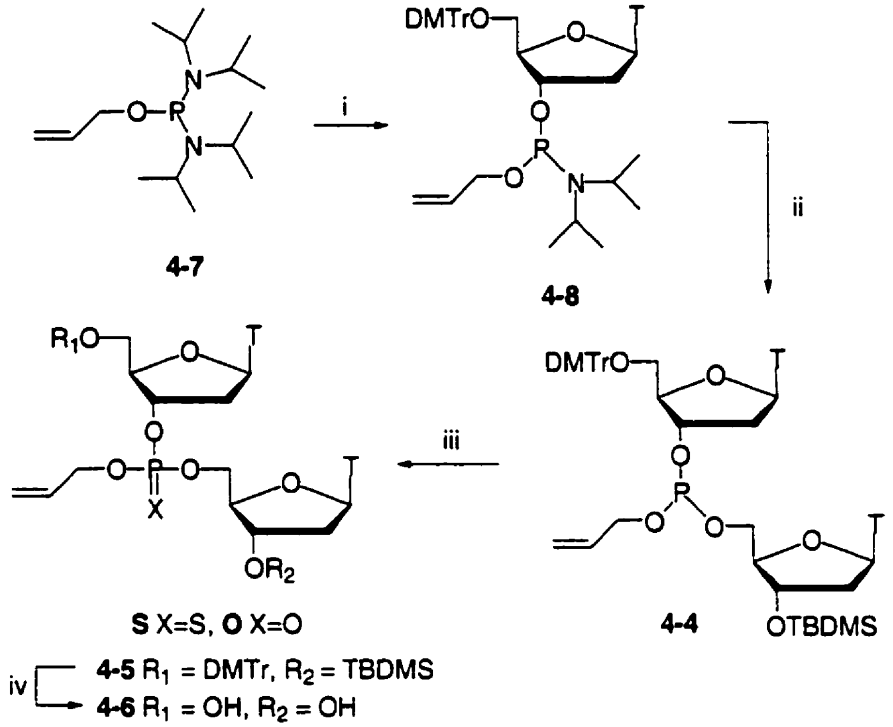


i. PCl_3, Pyridine, Et_2O, - 78 °C; ii. 5'-O-DMTr-thymidine, Pyridine, followed by 3'-O-TBDMS-thymidine, Pyridine; iii I_2 in THF and H_2O or Beaucage's Reagent; iv. 80 % Acetic Acid, then TBAF

Scheme 4.3: Synthesis of **4-6** via phosphite triester approach

In the phosphoramidite approach (Scheme 4.4), allyl-bis(diisopropylamino)phosphine **4-7** was treated with 5'-O-DMTr-thymidine, diisopropylamine and tetrazole in acetonitrile to give phosphoramidite **4-8**. It was then allowed to react with 3'-O-TBDMS-thymidine and tetrazole in acetonitrile to yield phosphite triester **4-4**, which was directly oxidized or sulfurized to provide phosphate **4-**

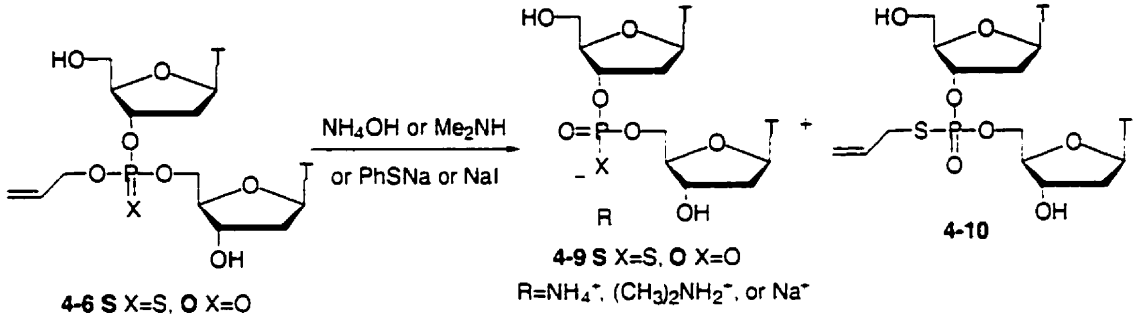
5O or phosphorothioate **4-5S**. The protecting groups were removed to furnish dithymidine **4-6O** or **4-6S** with the allyl protection on the internucleotide linkage.



i. 5'-O- DMTr-thymidine, diisopropylamine, tetrazole, CH_3CN; ii. 3'-O-TBDMS-thymidine, tetrazole; iii. I_2 in THF and H_2O or Beaucage's Reagent; iv. 80 % Acetic Acid, then TBAF

Scheme 4.4: Synthesis of **4-6** via phosphoramidite approach

4.3. Removal of the Allyl Groups by Nucleophiles



Scheme 4.5: Removal of the allyl protecting groups

With dinucleotides **4-6O** and **4-6S** in hand, we then proceeded to remove the allyl groups by nucleophiles. We initially used concentrated ammonia, 40% aqueous dimethylamine or thiophenolate as deprotecting reagents (Scheme 4.5). ^{31}P NMR clearly indicated the progress of the reactions. In the deprotection of **4-6O**, the original two peaks (-1.22 ppm, -1.37 ppm) of allyl phosphate **4-6O** became a single peak (-1.68 ppm). In the case of phosphorothioate **4-6S**, two peaks characteristic of the triesters at 68.49 ppm and 68.37 ppm moved to 55.95 ppm and 55.58 ppm after deprotection with the same reagents. The reaction time was approximately the same as for phosphate **4-6O**. The deprotected yields were quantitative as evaluated by ^{31}P NMR. The typical ^{31}P NMR spectra are shown in Figure 4.1. The deprotection results are summarized in Table 4.1.

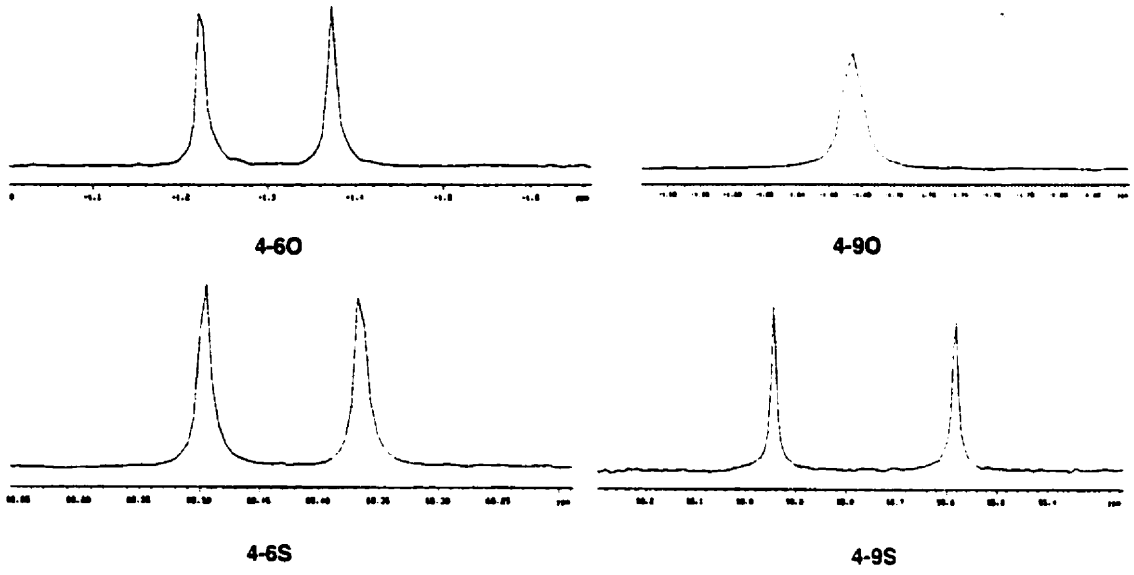


Figure 4.1: Typical ^{31}P NMR spectra of **4-6** and **4-9**

Table 4.1: Deprotection of the allyl groups by nucleophiles*

Nucleophiles	Temperature (°C)	Time (mins)	Yield (by ^{31}P NMR)
Conc. NH_4OH	55	120	100%
40% aq. $(CH_3)_2NH$	RT	30	100%
Aq. PhSNa	RT	10	100%

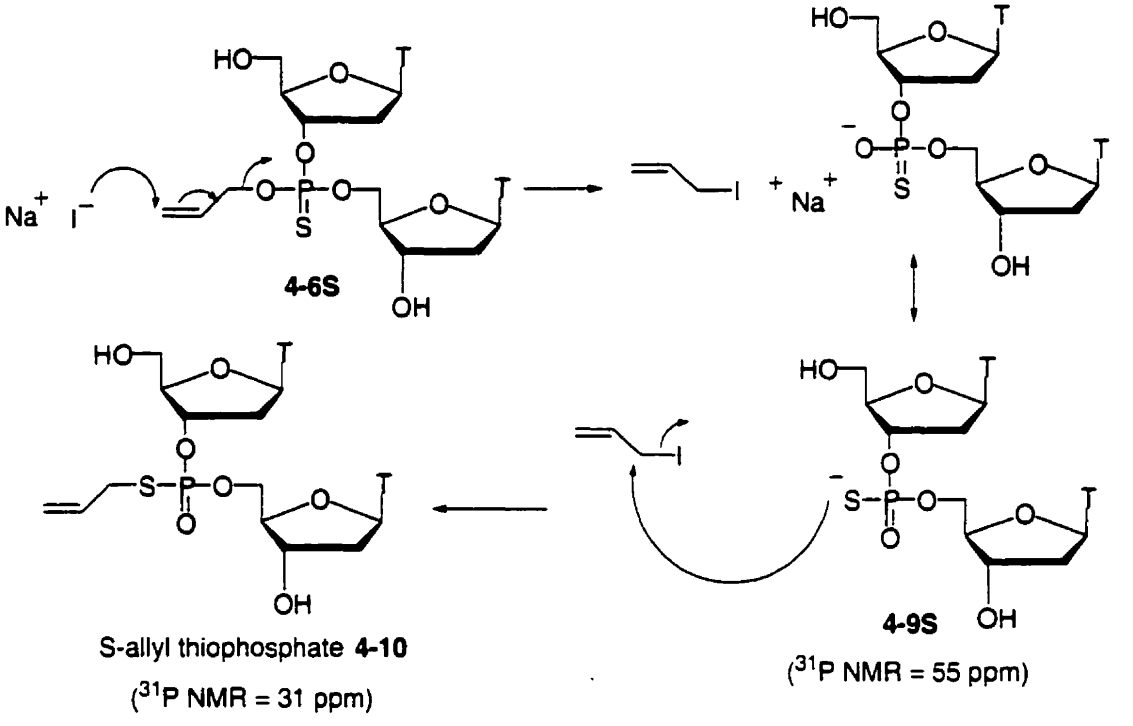
* The dinucleotides used for the deprotections were **4-6O** and **4-6S**, reaction time was approximately same for the deprotections of **4-6O** or **4-6S**.

We next studied the removal of the allyl groups under neutral conditions. Deesterification of phosphate triester could be achieved by use of sodium iodide.[209] When **4-6O** was treated with an aqueous solution of sodium iodide at 55 °C for 20 hours, phosphate **4-9O** and starting material **4-6O** were obtained in a ratio of 15:1. However, when phosphorothioate **4-6S** was treated with sodium iodide solution under the same reaction conditions, ^{31}P NMR showed the two peaks around 68 ppm were replaced by two sets of two peaks around 55 ppm and 31 ppm in a ratio of 3:1. These two sets are characteristic of the expected phosphorothioate **4-9S** and of the S-allyl thiophosphate **4-10**. The identity of the latter was confirmed by reaction of **4-9S** with allyl iodide. Similar results were obtained when acetone was used as a solvent. We propose the products were formed by the pathways as shown in Scheme 4.6.

In order to suppress side reactions, we used thiourea to trap the allyl iodide formed. With 50 equivalents of thiourea present in the system, **4-9O** was the only product

[209] Yannopoulos, C. Ph.D. thesis, McGill University, **1997**

formed in the deprotection of phosphate **4-6O**. In the case of the phosphorothioate, the ratio of **4-9S** to S-allyl thiophosphate **4-10** improved to 66:1.



Scheme 4.6: The formation of S-allyl thiophosphate

4.4. Applications of the Allyl Protections to the Solid Phase Synthesis of Oligonucleotides

We next applied our methods to the solid phase synthesis of oligonucleotides. P-*O*-allyl phosphoramidites with or without β-cyanoethyl phosphoramidites were used with automated DNA synthesizers to synthesize oligonucleotides of desired length and sequence. This work was carried out at ISIS Pharmaceuticals (Carlsbad, CA). Table 4.2 illustrates selected oligonucleotides that have been synthesized.

When an allyl protecting group is used in phosphodiester (P=O) oligonucleotide synthesis, it becomes necessary to use non-standard oxidation conditions. Traditionally, 0.1 M iodine in water/pyridine/THF (2/20/80 v/v/v) is used in automated DNA synthesizers. Although the allyl group can survive under such conditions, a clean oxidation reaction does not occur. To overcome this difficulty, we used 10-camphorsulphonyl oxaziridine, obtained from Aldrich, as a source of oxygen. A 0.5 M solution of the oxaziridine in acetonitrile gives a clean oxidation.

Table 4.2: Some oligonucleotides synthesized

	Sequence[1] (5'/3')	Backbone	Mass Observed	Mass Expected	HPLC[2] retention time
1	TTT TTT TTT TTT	P=S	3765.828	3764.53	27.01
2	TTT TTT TTT TTT	P=O	3589.828	3588.11	19.51
3	TGC ATC CCC CAGGCC ACC AT	P=S	6288.688	6286.58	22.65
4	TGC ATC CCC CAGGCC ACC AT	P=O	5984.688	5980.88	17.98
5	GAA CT	P=O	742.589	742.6	19.54
6	GAC CT	P=O	730.588	730.6	8.73
7	GAG CT	P=O	750.590	750.6	7.79
8	GAA CT	P=S	774.549	774.6	24.65
9	GAC CT	P=S	762.548	762.6	24.81
10	GAG CT	P=S	782.550	782.7	24.47
11	GCC CAA GCT ATCCGT GGC CA	P=O	6060.550	6062.13	22.48

12	GCC CAA GCT GGCATC CGT CA	P=S	6364.170	6365.73	26.95
13	GTA CT	P=O	738.088	738.0	17.27

[1] All underlined nucleosides (N) were derived from 3'-P-*O*-allyl phosphoramidite; [2]Conditions: Waters 600E with 991 detector; Waters C4 column (3.9X300 mm); Solvent A: 50 mM TEA-Ac, pH 7.0; Solvent B: acetonitrile; 1.5 mL/min. flow rate; Gradient: 5% B for first five minutes with linear increase in B to 60% during the next 55 minutes.

The allyl group is cleaved in a deblocking step using an aqueous solution of concentrated ammonium hydroxide having 2 % mercaptoethanol present in the solution. The mercaptan is presumably responsible for removal of the allyl group, while concentrated ammonium hydroxide deprotects the standard exocyclic amine protecting groups.

In conclusion, we demonstrated the removal of the allyl protective group in the internucleotide linkage of nucleotides can be achieved by nucleophiles. The protocol presenteded here can be used for routine solid phase synthesis of oligonucleotide phosphates and phosphorothioates and has been recently published.*

4.5. (Allyloxy)carbonyl (AOC) Protections for the Nucleoside Bases in Oligonucleotide Synthesis

To further explore the allyl protection, (allyloxyl)carbonyl (AOC) groups and allyl groups were used as the protectors for the nucleoside bases and internucleotide linkages, respectively. The syntheses of the four monomers were carried out in collaboration with Ms. Xiaoling Chen. These monomers were then used for the solid

* Manoharan, M.; Lu, Y.; Casper, M. D. and Just, G. *Org. Lett.* **2000**, 2, 243

phase synthesis of oligonucleotide phosphates and phosphorothioates. The preliminary results showed that the allyl and AOC groups were easily deblocked using the protocol described in Section 4.4. This work is in collaboration with Isis Pharmaceuticals and is still in progress.

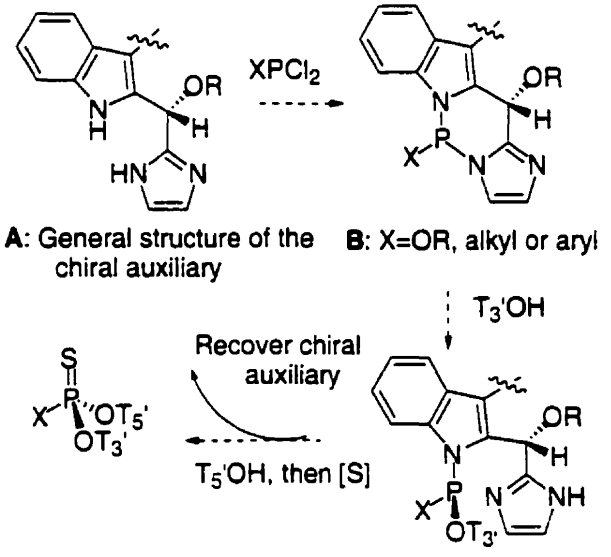
Chapter 5. Attempts to Develop a Recoverable Chiral Auxiliary for the Stereoselective Synthesis of Phosphorothioates and Alkyl Phosphinate Analogs

5.1. Plan of Study

The chiral auxiliaries which were previously synthesized in our laboratory[168,171,172,176] could be used for the stereoselective synthesis of phosphorothioates with a certain degree of success. None of those chiral auxiliaries could be recovered at the end of the synthesis. Our initial idea was to develop a recoverable chiral auxiliary which could lead to the diastereoselective synthesis of phosphorothioates. Preferably, it could also be used for synthesis of phosphonate derivatives.

In the imidazo-oxazaphosphorine approach (Scheme 1.16),[172] reaction of the chiral hydroxypropylimidazole **22** with ethyl dichlorophosphite and triethylamine gave a mixture of diastereomeric imidazophosphorines **23**. Heating the reaction mixture effected a presumably acid catalyzed epimerization to a single diastereoisomer of **23**. Reaction of the latter with a variety of alcohols converted **23** to the phosphite triester **24**, and hence to the corresponding phosphorothioate in a stereospecific manner. Extreme hydrolytic instability of **23**, and our inability to synthesize an analog of **23** in which the ethyl group is replaced by a nucleoside, convinced us to investigate a more stable azole, such as indole, as a leaving group.

As illustrated in the indolo-oxazaphosphorine approach (Scheme 1.17),[176] the reaction of hydroxyalkylindoles **26** with phosphorus trichloride provided the phosphorochloridite, which was converted to a mixture of diastereomeric indolophosphorines **27** by reaction with 5'-O-protected thymidine. The latter could not be equilibrated to a single diastereomer under basic or acidic conditions without decomposition. The indole residue could act as a leaving group, provided the nucleophilicity of the 5'-hydroxy group of thymidine was activated by a strong base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The displacement reaction proceeded in a stereospecific manner.



Scheme 5.1

It was therefore logical to link the two leaving groups by a chiral substituted methylene group. This would provide a recoverable chiral auxiliary suitable for the synthesis of both chiral phosphonates and phosphate analogs, as illustrated in Scheme

5.1. In addition, ring system **B** has never been synthesized before, and it therefore would be a worthwhile target for synthesis and evaluation of reactivity.

Two key issues needed to be addressed, which are whether the six-membered ring will be formed when such an auxiliary reacts with dichlorophosphorus compounds, and whether the imidazole and indole moieties will leave sequentially when reacted with nucleosides. We therefore synthesized achiral and racemic model compounds to test the feasibility of the methodology.

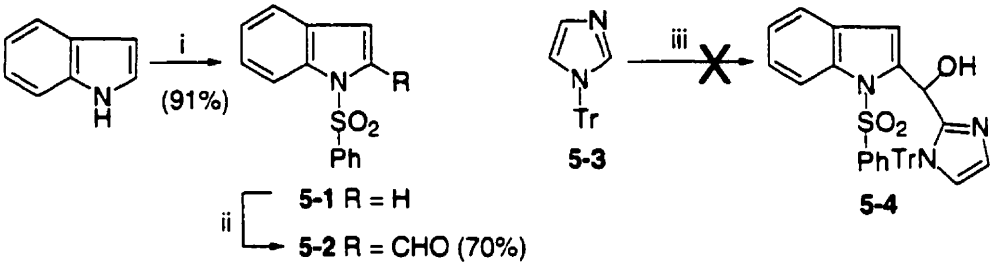
5.2. Model Studies

5.2.1. Synthesis of the Model Compounds

Our initial attempt is summarized in Scheme 5.2. Treatment of phenylsulfonyl protected indole **5-1** with *n*-butyllithium, followed by quenching with DMF, gave aldehyde **5-2**. N-trityl imidazole **5-3** was then reacted with *n*-butyllithium, and added to **5-2**. However, no desired coupling product corresponding to **5-4** was obtained, presumably due to the steric hindrance introduced by the two large protecting groups on indole and imidazole. In order to circumvent this problem, we carried out an analogous reaction with an unprotected indole (Scheme 5.3). Indole-2-carboxylic acid **5-5** was transformed via its acid chloride **5-6** to Weinreb amide[210] **5-7**, and the latter was treated with two equivalents of the N-tritylimidazole anion to give **5-8**. Deprotection gave achiral

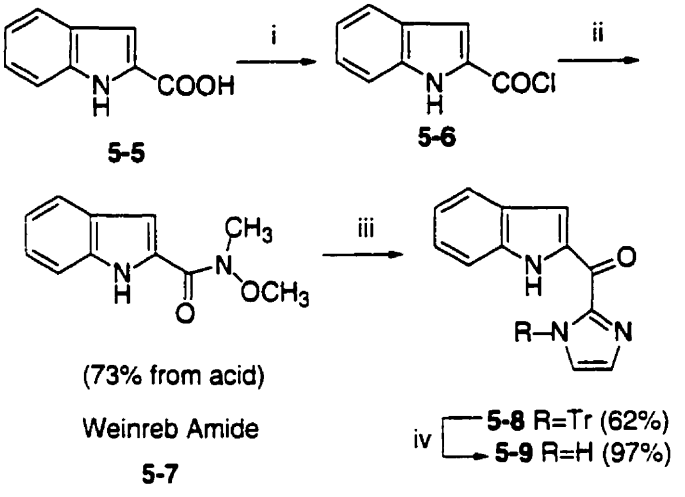
[210] Nahm, S. and Weinreb, S. M. *Tetrehedron Lett.* **1981**, 22, 3815

ketone **5-9**. Although not appropriate for the synthesis of a chiral phosphonate or phosphorothioate, we investigated the reaction of **5-9** with methyl dichlorophosphite to establish the stability and reactivity of cyclic phosphite derivative **5-10**.



i. NaH, $PhSO_2Cl$, THF, 0 °C to RT ii. *n*-BuLi, THF, -78 °C to RT then DMF;
iii. *n*-BuLi, THF, -78 °C to RT then added to **5-2**

Scheme 5.2: Attempt to synthesize model compound



i. $SOCl_2$, Et_3N, DME, 0 °C to RT ; ii. $CH_3NH(OCH_3)\cdot HCl$, CH_2Cl_2, 0 °C to RT;
iii. *n*-BuLi, N-trityl imidazole, THF, -78 °C to RT; iv. AcOH/MeOH. reflux

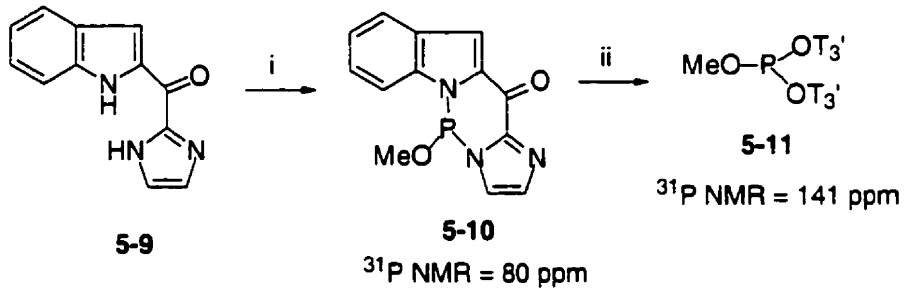
Scheme 5.3: Making model compound through Weinreb amide

5.2.2. Model Reactions

With achiral model compound **5-9** in hand, we then carried out model reactions (Scheme 5.4). Ketone **5-9** in freshly distilled dichloromethane containing triethylamine in a dry NMR tube was treated with methyl dichlorophosphite at 0 °C. ^{31}P NMR showed that the original resonance at around 180 ppm corresponding to methyl dichlorophosphite disappeared and a new resonance at 80 ppm corresponding to **5-10** appeared immediately. 5'-O-*tert*-butyldimethylsilyl thymidine (T_3·OH) dissolved in dichloromethane was then added to the reaction mixture. In contrast to imidazo-oxazaphophorine,[172] the reaction went slowly. After allowing the reaction to proceed at ambient temperature for about 15 hours, the original resonance at 80 ppm disappeared and a new resonance at 141 ppm was observed. The product turned out to have structure **5-11**. That meant thymidine not only displaced the imidazole moiety, but also displaced the indole moiety. Since indole is not a good leaving group under such mild conditions, we reasoned that the conjugation of indole with the adjacent electron withdrawing carbonyl group made it a better leaving group.

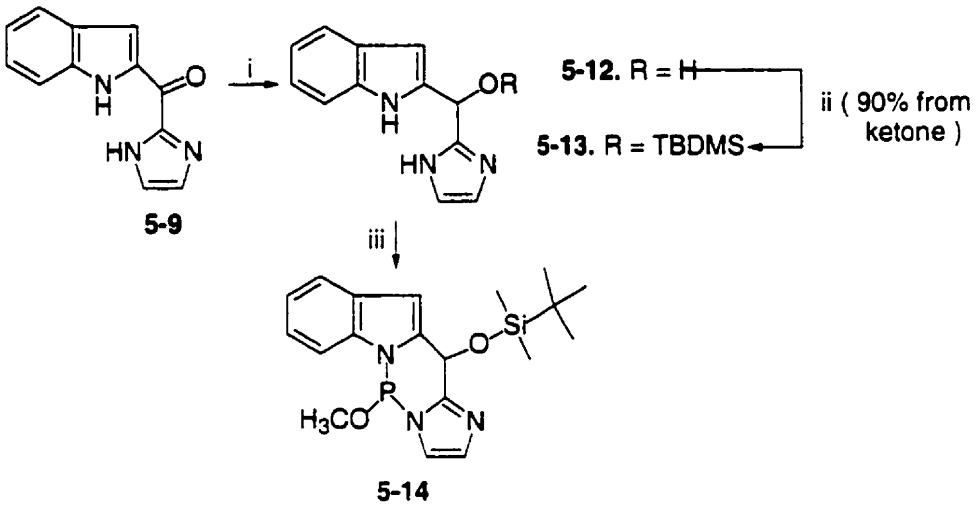
Having established that ring systems of type **5-10** are readily formed, we then proceeded to reduce the ketone **5-9** to alcohol **5-12**, which was protected as its TBDMS ether **5-13**. When **5-13** was treated with methyl dichlorophosphite, ^{31}P NMR revealed two resonances at 88.6 ppm (major) and 80.3 ppm (minor). After heating at 55 °C to equilibrate those two products, the resonance at 88.6 ppm shifted to 80.3 ppm. Without purification, T_3·OH in dry dichloromethane was added into the solution of **5-14** in the NMR tube. No reaction happened initially. The solution was allowed to stand overnight

at room temperature. A reaction started to occur, but was very sluggish and after several days, less than 10% of **5-14** had reacted. In contrast to the normally very reactive and therefore unstable imidazo-oxazaphosphorine intermediate, the cyclic compound **5-14** could be separated by silica gel column chromatography and fully characterized.



i. $MeOPCl_2$, 2.2 eq Et_3N, CH_2Cl_2, 0 °C to RT; ii. T_3OH, CH_2Cl_2, RT

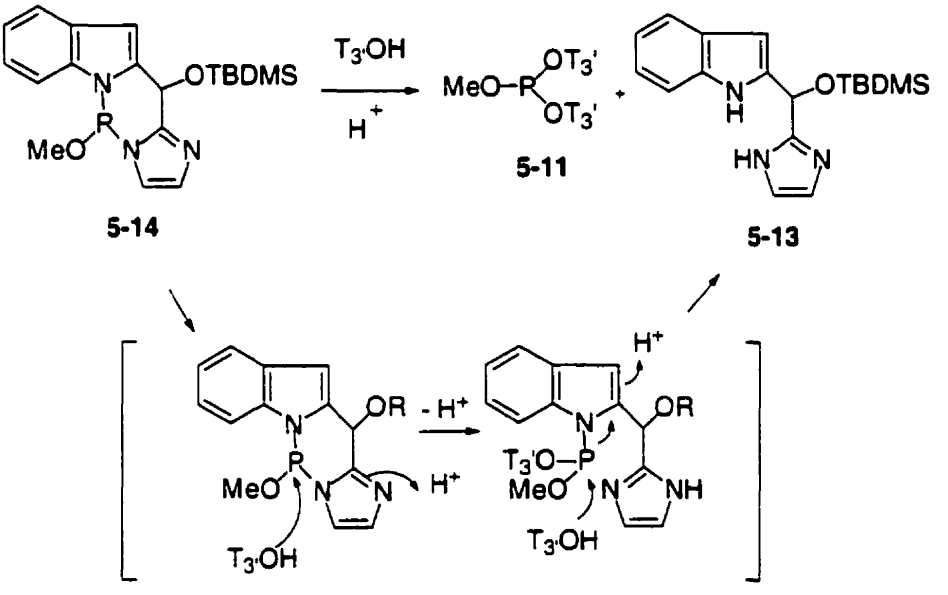
Scheme 5.4: Model reactions with **5-9**



i. $LiAlH_4$, THF, 0 °C to RT; ii. TBDMSCl, imidazole, DMF; iii. $MeOPCl_2$, 2.2 eq Et_3N, CH_2Cl_2

Scheme 5.5

It is known that the displacement of imidazole can be facilitated under acidic conditions. We therefore tried to displace the imidazole moiety in the presence of acid. Dry acetic acid was added along with T_3·OH to **5-14** in dichloromethane. Phosphite triester **5-11** was obtained after purification. This result again indicated a double displacement process as shown in Scheme 5.6.

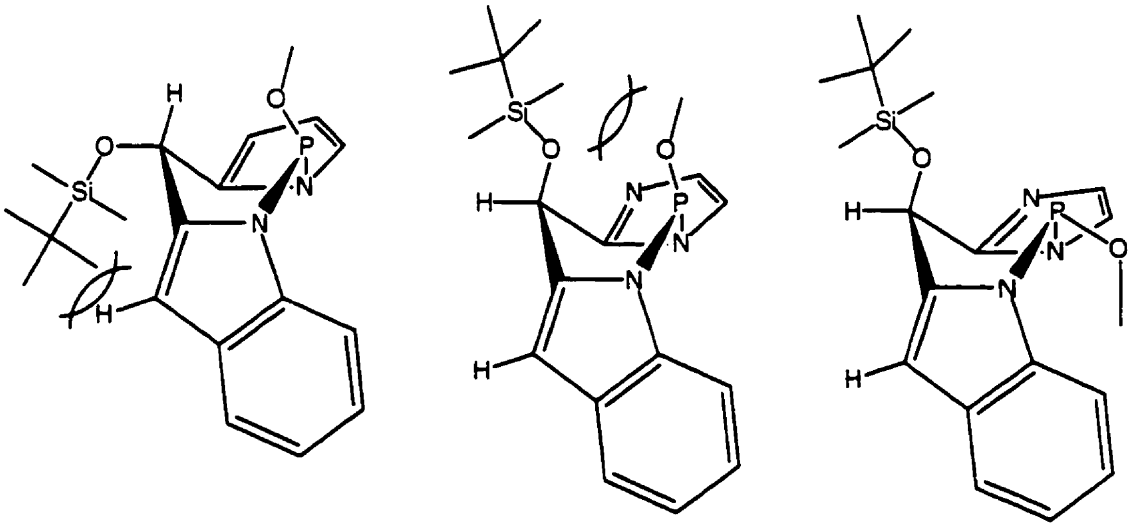


Scheme 5.6: Displacements under acidic conditions

We suspected that the lack of reactivity of **5-14** was due to steric hindrance, and thus carried out molecular modeling to establish the most stable conformation of **5-14**. The calculations were done by applying MacroModel program (version 5.5). Different structures were built and pre-minimized first, then Monte-Carlo searches were carried out to find out the global minima of different molecules. Cyclic compound **5-14** equilibrated

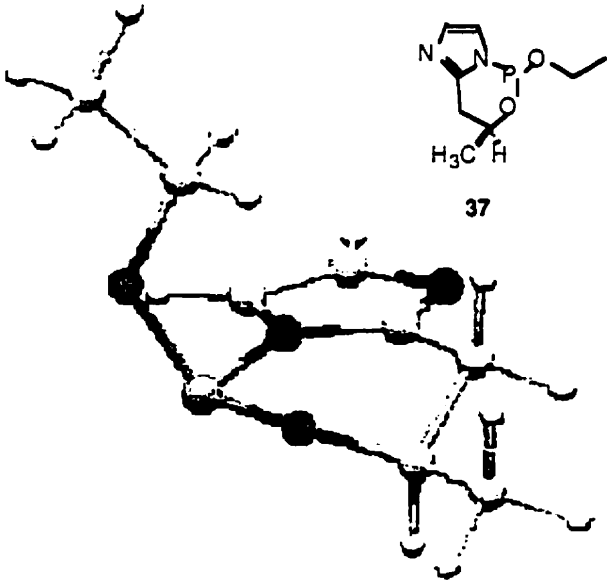
to give the thermodynamically more stable conformation. For the related systems,[185,195,196] this had meant that an exocyclic methoxy group is axially or pseudoaxially disposed, whereas the bulky silyloxy group prefers an equatorial arrangement. Depending if the phosphorus containing six-membered ring adopts a boat or chair-like conformation, the two substitutents have a *trans* or *cis* relationship. Much to our surprise, calculation indicated that the most stable conformation was a boat, in which the TBDMS group was axially disposed and the methoxy group took up the pseudo-equatorial conformation (**5-14b**). This is presumably due to the fact that the hydrogen attached to the 3-position of indole exerts enough steric hindrance (**A**) to make the relatively unhindered axial conformation an energy minimum, in which the only interaction of any consequence is a flag-pole interaction with the phosphorus lone pair. Calculation also indicated that conformer **5-14b** is about 4 kcal/mole more stable than conformer **5-14a**. This conformation also explains why the displacement of the imidazole is so slow, since the approach of a nucleophile is blocked by the bulky OTBDMS group. Scheme 5.7 shows these conformers.

For comparison, imidazo-oxazaphosphorine **37** was also built up and minimized. Figure 5.1 shows the molecular modeling result. Since the phosphorus atom in conformer was easily accessed by nucleophile, the reaction of **37** with thymidine was very fast.



A **B (5-14a)** **C (5-14b)**

Scheme 5.7: The best conformation (**C**) of **5-14**

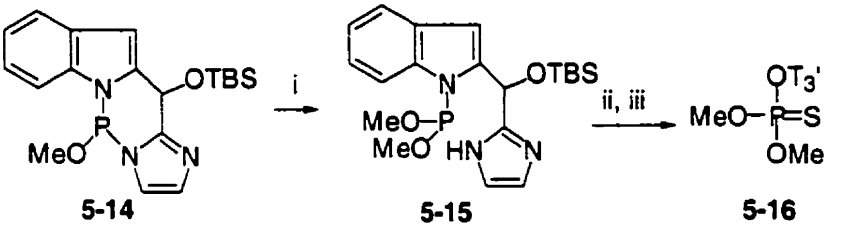


37

Figure 5.1

In order to confirm that steric hindrance was the cause for the slow reaction of **5-14** with T_3·OH, we repeated the reaction with a smaller alcohol. Upon the addition of

methanol to a solution of **5-14** in dichloromethane, ^{31}P NMR revealed the new peak within 20 minutes at around 146 ppm which was assigned to dimethyl phosphite **5-15**. When **5-15** was treated with T_3·OH in the presence of DBU, a further displacement occurred. The ^{31}P NMR shifted to 140 ppm, indicating the formation of phosphite triester. Sulfurization by Beaucage's reagent yielded phosphorothioate **5-16** (Scheme 5.8).



5-14 **5-15** **5-16**

i. MeOH, RT; ii T_3·OH, DBU, CH_2Cl_2, RT; iii. Beaucage's reagent

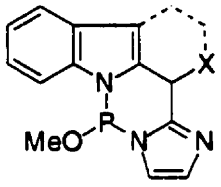
Scheme 5.8: Displacement by methanol

So far, we have demonstrated that the new ring system incorporating imidazole and indole can be readily formed. The displacements of imidazole and indole moieties by alcohols occurred efficiently provided there was no big steric hindrance. The use of a structurally less hindered chiral system derived from tryptophan will be discussed in the following section.

5.3. Chiral Auxiliary Derived from Tryptophan

Having demonstrated that that six-membered ring system A incorporating the heteroatoms of an indole and imidazole connected to a trivalent phosphorus can be readily

formed, we then investigated whether the displacements of imidazole and (base catalyzed) indole could be carried out sequentially in a stereoselective and expeditious manner. We decided to substitute the X group in **A** (X= OTBDMS) by a ring (see dotted lines).



A

An easy way to construct such a ring system containing a chiral center is based on the Pictet-Spengler reaction of a tryptophan derivative with an appropriate aldehyde, as outlined in Scheme 5.9. Imidazole 2-carboxaldehyde **5-18** was prepared according to the literature procedure.[211] Reductive amination of the commercially available L-tryptophan methyl ester **5-17** with benzaldehyde provided benzyl protected derivative **5-19**. Reaction of **5-19** and aldehyde **5-18** in the presence of *p*-toluenesulfonic acid gave the desired product as one diastereomer after column purification. The reported[212,213,214] uncatalyzed reaction proceeded in an unsatisfactory manner.

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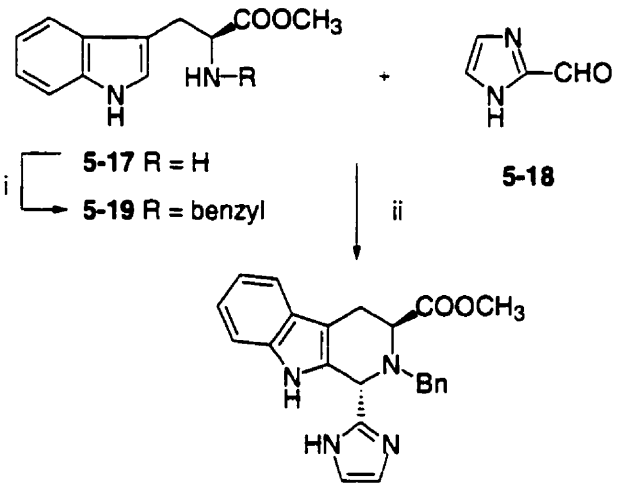
[211] Kirk, K. L. *J. Org. Chem.* **1978**, 43, 4381

[212] Cox, E. D.; Hamaker, L. K.; Li, J.; Yu, P.; Czerwinski, K. M.; Deng, L.; Bennett, D. W.; Cook, J. M. *J. Org. Chem.* **1997**, 62, 44

[213] Ungemach, F.; DiPierro, M.; Weber, R. and Cook, J. M. *J. Org. Chem.* **1981**, 46, 164



5-20

i. benzaldehyde, MeOH, $NaCNBH_3$; ii. *p*-TsOH, Benzene, reflux, Dean-Stark water trap

Scheme 5.9: Chiral auxiliary derived from tryptophan

Cook and coworkers[213,215] have shown Pictet-Spengler reaction of N_b-benzyltryptophan methyl ester with aldehydes of large steric size led to the stereospecific formation of *trans* diastereomers. The stereochemistry of the chiral auxiliary **5-20** was assigned accordingly.

5.4. Stereoselective Synthesis of Phosphorothioates

Chiral auxiliary **5-20** was used for the stereoselective synthesis of phosphorothioates, as outlined in Scheme 5.10.

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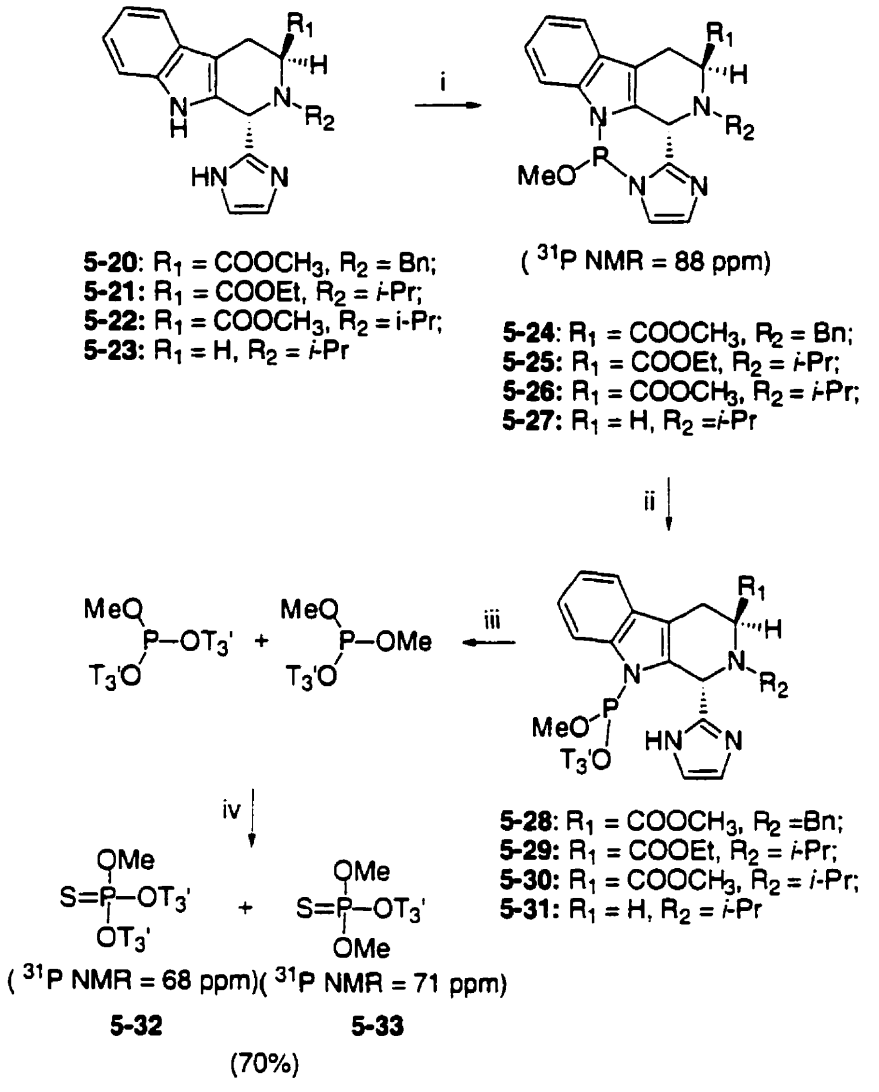
[214] Soerens, D.; Sandrin, J.; Ungemach, F.; Mokry, P.; Wu, G. S.; Yamanaka, E.; Hutchins, L.; DiPierro, M. and Cook, J. M. *J. Org. Chem.* **1979**, 44, 535

[215] Czerwinski, K. M.; Deng, L. and Cook, J. M. *Tetrahedron Lett.* **1991**, 33, 4721



5-20: R_1 = $COOCH_3$, R_2 = Bn;
5-21: R_1 = COOEt, R_2 = *i*-Pr;
5-22: R_1 = $COOCH_3$, R_2 = i-Pr;
5-23: R_1 = H, R_2 = *i*-Pr

(^{31}P NMR = 88 ppm)

5-24: R_1 = $COOCH_3$, R_2 = Bn;
5-25: R_1 = COOEt, R_2 = *i*-Pr;
5-26: R_1 = $COOCH_3$, R_2 = *i*-Pr;
5-27: R_1 = H, R_2 = *i*-Pr

5-28: R_1 = $COOCH_3$, R_2 = Bn;
5-29: R_1 = COOEt, R_2 = *i*-Pr;
5-30: R_1 = $COOCH_3$, R_2 = *i*-Pr;
5-31: R_1 = H, R_2 = *i*-Pr

(^{31}P NMR = 68 ppm) (^{31}P NMR = 71 ppm)

5-32 **5-33**

(70%)

i. $MeOPCl_2$, 2.2 eq Et_3N, CH_2Cl_2 or $CDCl_3$; ii 3.0 eq 5'-O-TBDMS-thymidine in CH_2Cl_2; iii. 55 °C, 15 hrs; iv. Beaucage's reagent

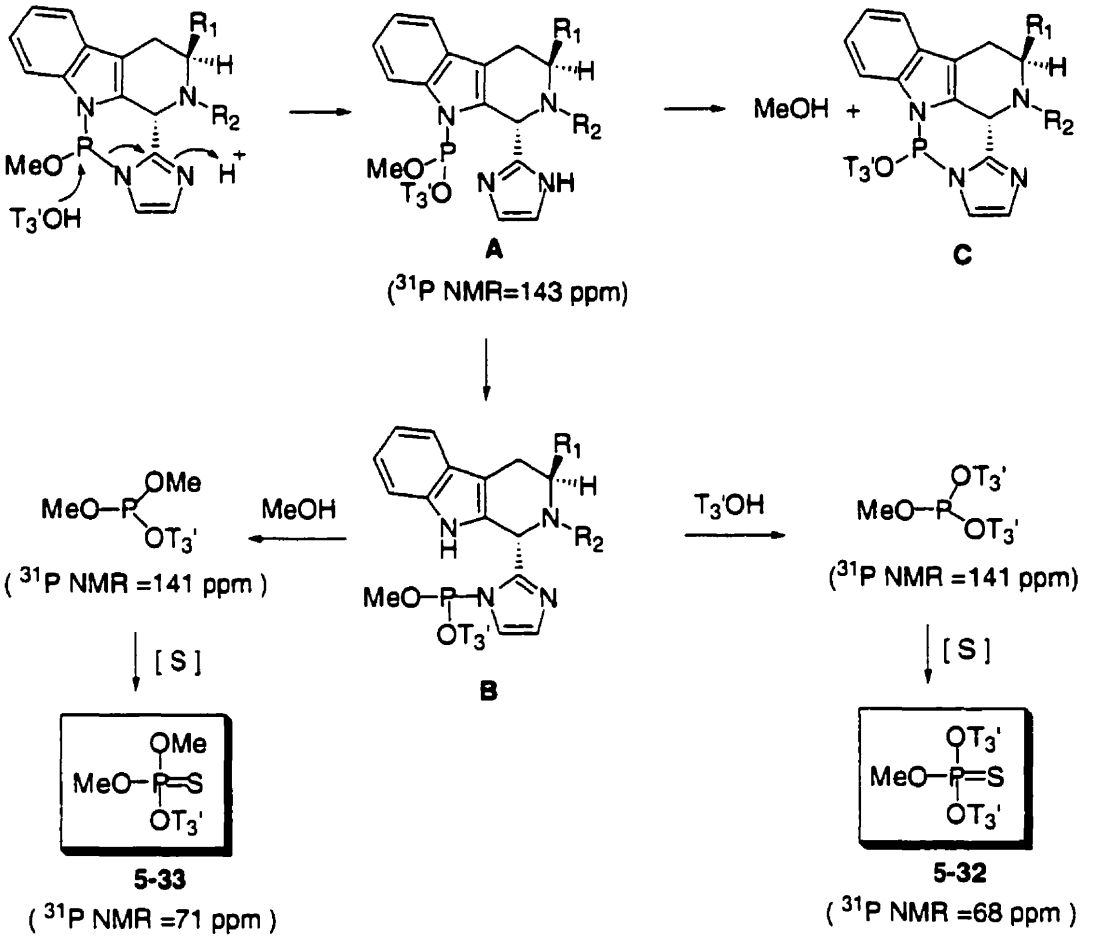
Scheme 5.10

Reaction of **5-20** with methyl dichlorophosphite and triethylamine in dichloromethane was carried out in a dry NMR tube. ^{31}P NMR revealed the appearance of a single new peak at 88 ppm, corresponding to the formation of **5-24** as a single

diastereomer. Addition of one equivalent of T_3·OH resulted in the rapid formation of **5-28** (^{31}P NMR = 143 ppm), accompanied by the formation of a small amount of material with ^{31}P NMR at 141 ppm. We suspected that the latter material was the product of a double displacement, and therefore repeated the reaction with an excess of T_3·OH. The major product **5-28** (^{31}P NMR = 143 ppm) formed immediately. Heating the mixture at 55 °C for 15 hours gave exclusively material with ^{31}P NMR at 141 ppm. Sulfurization with Beaucage's reagent proceeded as expected to give materials having the characteristic thiophosphate triester ^{31}P NMR resonances at around 70 ppm. Surprisingly, it consisted of a mixture of the expected dithymidyl thiophosphate **5-32**, accompanied by up to 25% of the dimethoxy thiophosphate **5-33**, which were separated by flash chromatography.

Rigorous exclusion of possible contamination by changing the solvent used, and running the reactions on the ethyl ester **5-21**, N_b-isopropyl auxiliary **5-22** and descarbomethoxy auxiliary **5-23** established that the source of methanol necessary to form the dimethoxy derivative **5-33** was the phosphorus attached methoxy group.

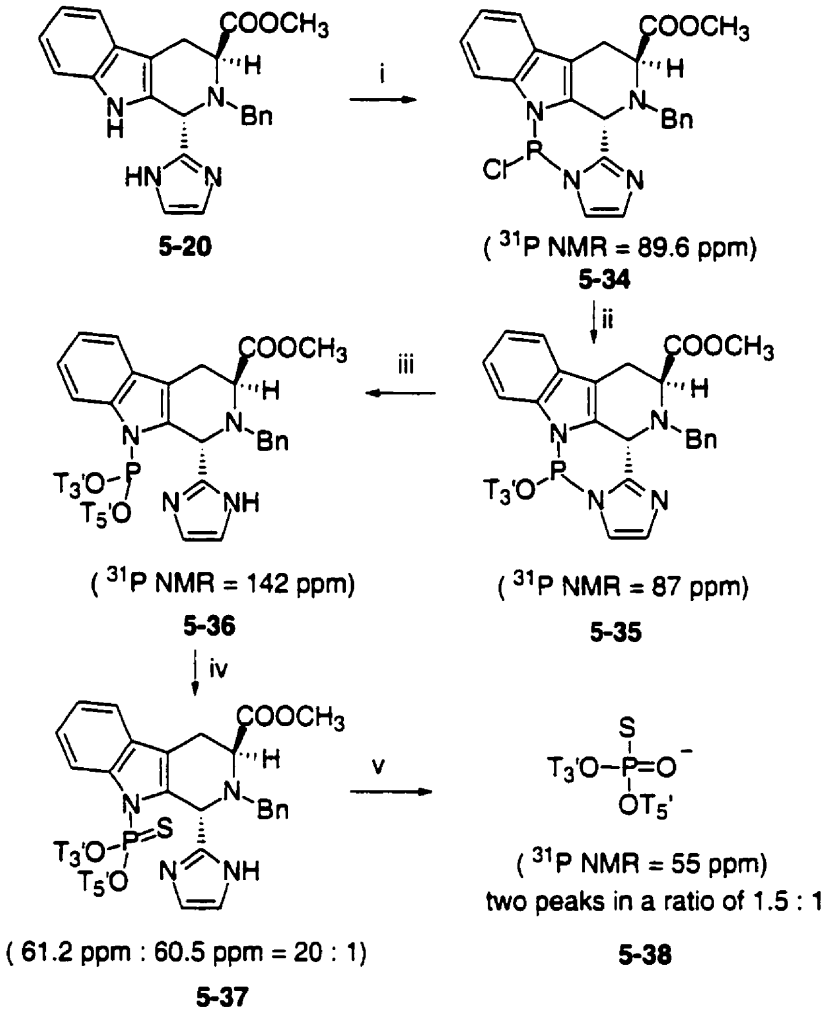
We proposed the reaction mechanism as depicted in Scheme 5.11. T_3·OH first displaces imidazole to yield a species of type **A**. Since imidazole is a good nucleophile, it can displace the methoxy group to form methanol and **C**. Alternatively, the displacement of indole by imidazole then gives **B**. Reactions of **B** with either T_3·OH or methanol, followed by sulfurization would give phosphorothioate **5-32** or **5-33**.



Scheme 5.11: Proposed mechanism

The problem was partly resolved in the following manner (Scheme 5.12). Reaction of chiral auxiliary **5-20** with phosphorus trichloride in dichloromethane containing triethylamine gave a 10:1 mixture of diastereomeric chlorophosphites **5-34** with ^{31}P NMR at about 89.6 ppm. Addition of T_3-OH gave **5-35** which had a ^{31}P MMR resonance around 87 ppm, typical for this type of compound. Addition of 3'-O-TBDMS-thymidine (T_5-OH) in dichloromethane provided **5-36** within 10 minutes, with a ^{31}P NMR signal at 142 ppm. Virtually no double displacement happened at this stage. Sulfurization with Beaucage's reagent and isolation gave the novel

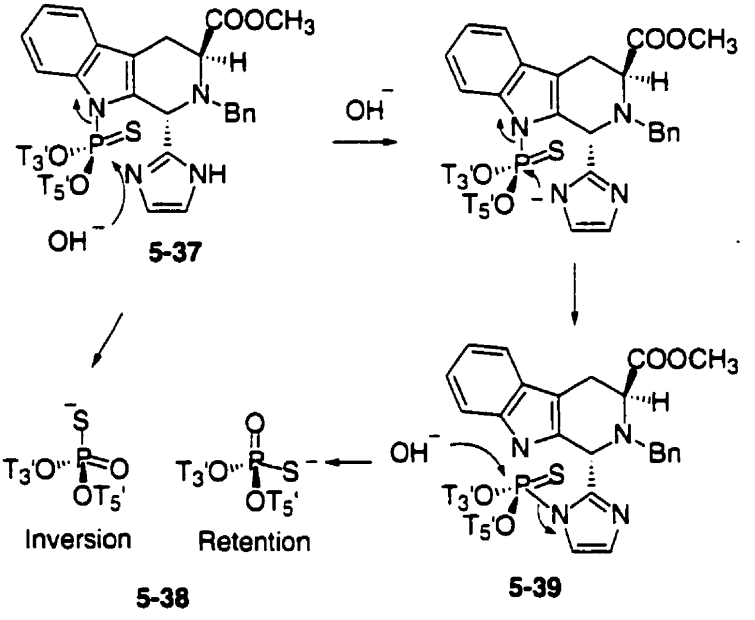
indolophosphorothioate **5-37**, with ^{31}P NMR at 61 ppm, as a mixture of diastereomers in a ratio of 20:1.



Scheme 5.12: Stereoselective synthesis of indolophosphorothioate **5-37**

The final step was the removal and recovery of chiral auxiliary at the end of the synthesis. Indolophosphorothioate **5-37** was treated with N-benzyltrimethylammonium hydroxide (triton B). Unfortunately, the diastereoselectivity we had so far got lost after

the removal of the chiral auxiliary, and the two diastereomers of **5-38** were obtained in a ratio of 3 to 2. This is again most likely due to competing single and double displacements, as outlined in Scheme 5.13. Direct displacement of the indole moiety by hydroxide would yield one diastereomer of **5-38**. Displacement of the indole moiety by imidazole anion, followed by displacement of imidazole by hydroxide would generate another diastereomer. Our attempts to protect the imidazole nitrogen are discussed in Section 5.5.



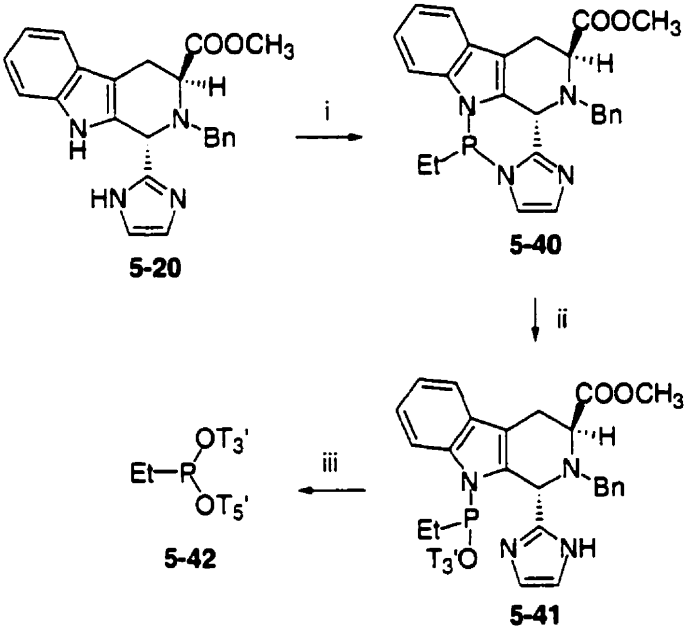
Scheme 5.13: Epimerization process

Although we were not able to obtain phosphorothioate stereoselectively using this method, we did obtain a novel indolophosphorothioate **5-37** in a highly stereoselective manner. This type of compound can be a very interesting P-chiral DNA analog since the

indole derivative moiety increases the lipophilicity of the nucleotide analog which can be important for antisense agents.

5.5. Stereoselective Synthesis of Alkyl Phosphinates

Chiral auxiliary **5-20** was applied to the stereoselective synthesis of alkyl phosphinates as shown in Scheme 5.14.



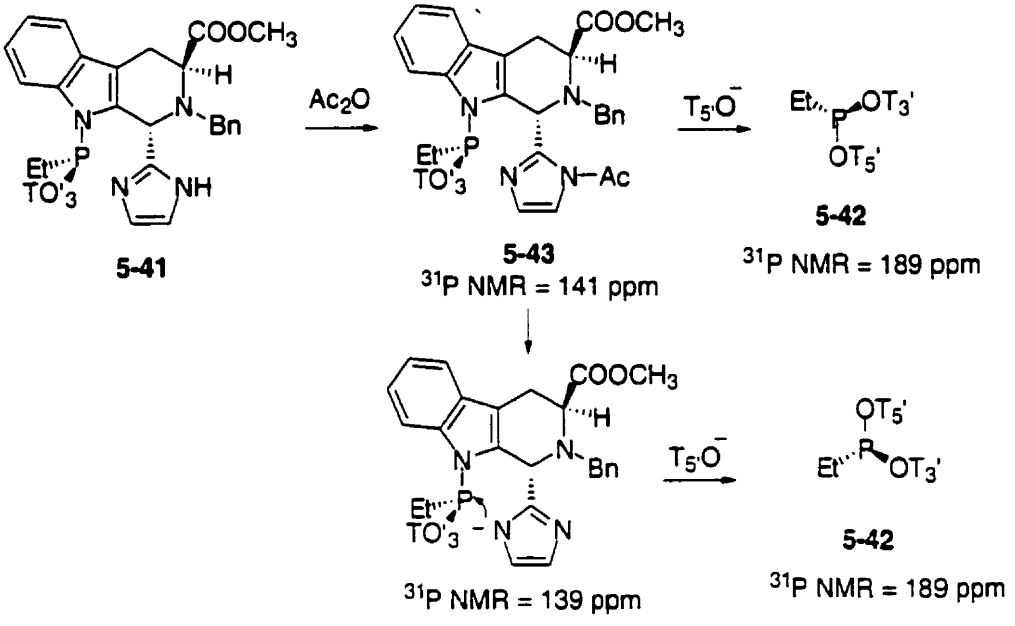
i. $EtPCl_2$, 2.2 eq Et_3N, CH_2Cl_2; ii 1.0 eq 5'-O-TBDMS-thymidine in CH_2Cl_2; iii. DBU, 3'-O-TBDMS-thymidine.

Scheme 5.14: Synthesis of alkyl phosphinates

Chiral auxiliary **5-20** in dry dichloromethane containing triethylamine was added to dichloroethyl phosphine in dry dichloromethane at –78 °C, and the mixture was warmed up slowly to room temperature. Intermediate **5-40** was formed immediately, with

^{31}P NMR resonances at 76.42 ppm and 74.98 ppm, in a ratio of 1 to 20. Then T_3·OH was added slowly at –78 °C. The mixture was allowed to warm up to room temperature. ^{31}P NMR showed two new resonances at 142.55 ppm and 142.04 ppm in a ratio of 20 to 1. Purification by flash chromatography gave alkyl phosphinamidite **5-41** as a single diastereomer. Stereospecific displacement of the indole moiety with T_5·OH and DBU in dichloromethane was not successful, and a mixture of epimeric alkylphosphinates **5-42** were obtained. The epimerization process is most likely due to double displacement, as described in scheme 5.13.

It seemed that in both the phosphorothioate and the alkyl phosphonate synthesis, the stereoselectivity got lost when imidazole participated to induce double displacement. We then attempted to protect imidazole nitrogens in **5-37** and **5-41**.



Scheme 5.15: Protecting imidazole nitrogen by acylation

Acetic anhydride was added to **5-41**, the mixture was stirred for about 5 minutes and the solvent was evaporated to provide the desired acetylated product **5-43**. When **5-43** was treated with T_5-OH in the presence of DBU, the original resonance at 141 ppm moved to 139 ppm and 189.7 ppm as the reaction proceeded. This clearly indicated that both the displacement of the indole moiety of **5-43** and deblocking of acyl protecting group occurred at the same time. Till the end of the reaction, the diastereoselectivity got lost and two epimers of **5-42** were obtained. Scheme 5.15 summarized this attempt.

We next tried to protect imidazole nitrogen by using groups stable to basic conditions. The trityl group is a typical protecting group for imidazole. However, treated **5-41** with either trityl chloride in the presence of triethylamine or with tritylpyridinium fluoroborate[216] did not yield the desired protected product, presumably due to the steric hindrance. Introducing 2-(trimethylsilyl)ethoxymethyl (SEM)[217] using standard procedure led to the formation of many products.

The methyl group was our next choice. Although imidazole was known to be a good nucleophile, reacting **5-41** with iodomethane directly failed to yield the desired product, and only starting material was recovered. Methylation could also have occurred in the presence of silver(I) oxide, so **5-41** was treated with iodomethane in the presence of silver(I) oxide in refluxing acetonitrile. The reaction went to completion in three hours. The product had ^{31}P NMR around 34 ppm, indicating some change at the phosphorus center, therefore this method was then also ruled out. Our next effort was to trap the

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[216] Hanessian, S. and Staub, A. P. A. *Tetrahedron Lett.* **1973**, 37, 3555
[217] Manoharan,, T. S. and Brown, R. S. *J. Org. Chem.* **1988**, 53, 1107

imidazole anion *in situ*. When cyclic intermediate **5-40** was formed, iodomethane was added to the reaction mixture along with T_3·OH, hoping imidazole anion could be trapped by iodomethane to block the nitrogen. However, only a complicated unidentified mixture was obtained at the end of the reaction.

Similarly, our attempts to protect the imidazole nitrogen of **5-37** were also unsuccessful.

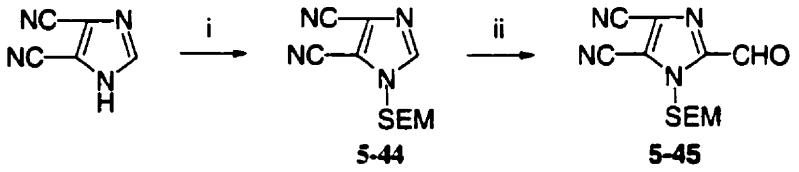
5.6. Chiral Auxiliary Incorporating Indole and Tetrazole

The imidazole induced double displacement seemed to be the cause for the epimerization process in both phosphorothioate and alkyl phosphonate synthesis. An alternative approach to solve this problem would be the use of other more acidic and less nucleophilic heterocyclic compounds instead of imidazole.

4,5-Dicyanoimidazole (pK_a = 5.37) and tetrazole (pK_a = 4.8) have much lower pK_a values than imidazole. We then tried to synthesize 4,5-dicyanoimidazole or tetrazole aldehyde, which can condense with tryptophan via Pictet-Spengler reaction to make the desired chiral auxiliaries.

We initially attempted to synthesize 4,5-dicyanoimidazole 2-carboxyaldehyde **5-45**, as shown in Scheme 5.16. 4,5-Dicyanoimidazole was first protected as its SEM derivative **5-44**. *n*-Butyllithium was added to **5-44** in THF at −78 °C to deprotonate, followed by quenching with DMF. However, the desired product **5-45** was not obtained, and only starting material was recovered. When the reaction was repeated with LDA, the same result was obtained. Suspecting the anion formation was not completed, we used

deuterium oxide instead of DMF to quench the reaction, and deuteriated **5-44** was obtained in over 90% yield.



5-44 **5-45**

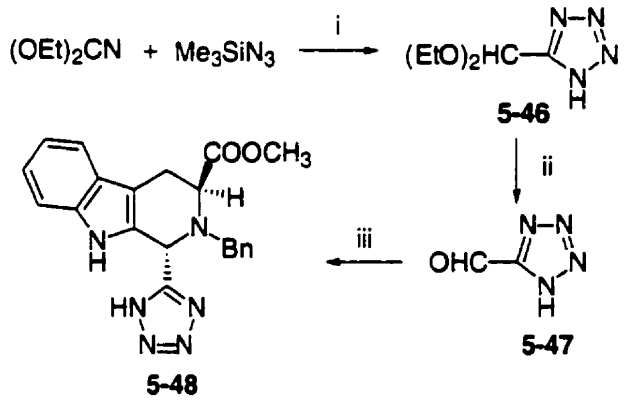
i. NaH, SEMCl, THF, 0 °C to RT; ii. n-BuLi, THF, DMF or D_2O, -78 °C to RT

Scheme 5.16: Synthesis of 4,5-dicyanoimidazole aldehyde

We next synthesized tetrazolocarbonaldehyde **5-47**. Its preparation[218] by the cyclization of diethoxylacetonitrile with hydrazoic acid in the presence of pyridine has been described in the literature. Due to the highly toxic nature of the hydrzaoic acid, we decided to use trimethylsilyl azide[219,220] as a replacement. Diethoxyacetonitrile and trimethylsilyl azide were stirred at 40 °C in a sealed vessel for 2 days. After work-up, the crude **5-46** was then treated with hydrochloric acid (1N) to hydrolyze the acetal. The aldehyde **5-47** and N_b-benzyl-L-tryptophan methyl ester were refluxed in toluene with a Dean-Stark water trap to furnish the desired chiral auxiliary **5-48**. The cyclization reaction between silyl azide and the nitrile proceeded in a moderate yield (~50%). Curran and

[218] Moderhack, D. *Chem. Ztg.* **1977**, 101, 403
[219] Nishiyama, K.; Yamaguchi, T. *Syn.* **1987**, 106
[220] Guy, A.; Lemor, A.; Doussot, J. and Lemaire, M. *Syn.* **1988**, 900

coworkers reported[221] the cyclization between tin azide and nitrile generally gave good yields. However, tin azide did not improve our reaction.



i. $CHCl_3$, pyridine, 40 °C, 3 days; ii. 4 N HCl;
iii. N_b-benzyl-L-tryptophan methyl ester, toluene, Dean-Stark, reflux, 10 hrs.

Scheme 5.17: Synthesis of chiral auxiliary incorporates indole and tetrazole moieties

The use of **5-48** for the stereoselective synthesis of alkyl phosphonate is illustrated in Scheme 5.18. Chiral auxiliary **5-48** was added to ethyl dichlorophosphine in dichloromethane containing triethylamine in a NMR tube at 0 °C. After the addition, ^{31}P NMR showed a single resonance at 82.2 ppm, which was assigned to cyclic intermediate **5-49**. After the treatment with T_3·OH, a new resonance at 142 ppm, which is typical for **5-50**, was observed. Without purification, T_5·OH and DBU in dichloromethane were added to the reaction mixture. Unfortunately, two peaks in a ratio of 2 to 1 at 189.8 ppm and 187.1 ppm characteristic for alkylphosphinites were observed. We then tried to isolate intermediate **5-50** by flash chromatography. A species with a ^{31}P NMR at 33 ppm was obtained after the purification. This work did not proceed further.

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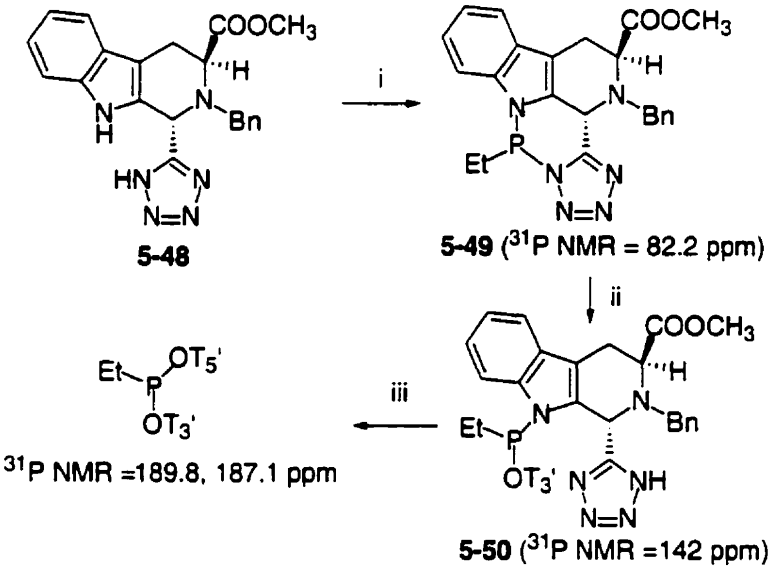
[221] Curran, D. P.; Hadida, S. and Kim, S. *Tetrahedron* **1999**. 55. 8997

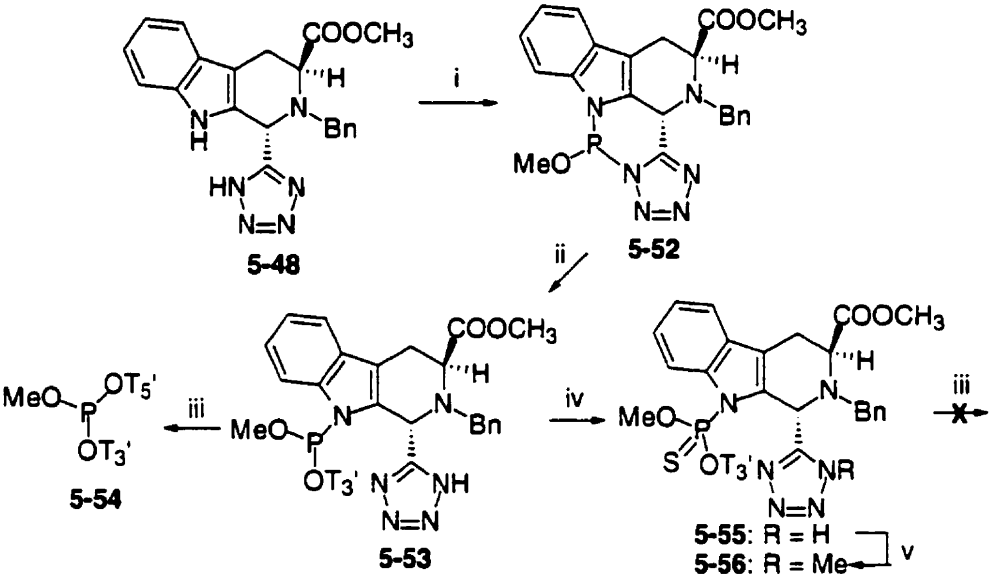


i.$EtPCl_2$, Et_3N, CH_2Cl_2, 0 °C; ii. T_3·OH; iii. T_5·OH, DBU

Scheme 5.18: Attempts to synthesize alkyl phosphonate

The stereoselecive synthesis of phosphorothioate from chiral auxiliary **5-48** is depicted in Scheme 5.19. After treatment of **5-48** with methyl dichlorophoshite in dichloromethane in the presence of triethylamine, ^{31}P NMR indicated a single resonance at 90.1 ppm, corresponding to the intermediate **5-52**. Further reaction with T_3·OH shifted the ^{31}P NMR to 140.8 ppm, which was assigned to **5-53**. The displacement of indole with T_5·OH in the presence of DBU did not proceed stereoselectively. Peaks at 140.6 ppm and 140.4 ppm, characteristic of phosphite triesters, were obtained in a ratio of 3 to 1. Suspecting the participation of tetrazole could be the source of epimerization, we then took an alternative approach. Indolophosphite triester **5-53** was sulfurized to **5-55**, then reacted with diazomethane to afford **5-56**, obtained as a single diastereomer after the

purification. We then treated **5-56** with T_5·OH and DBU and were disappointed to find out that the displacement did not occur, even after one day at 50 °C.



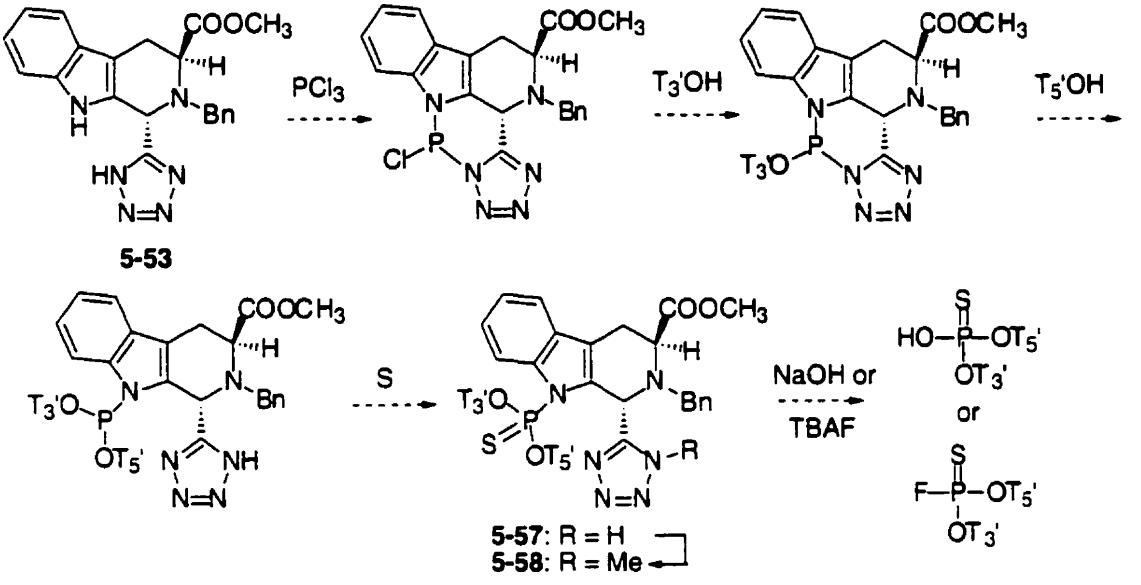
i. $MeOPCl_2$, Et_3N, CH_2Cl_2, 0 °C; ii. T_3·OH; iii. T_5·OH, DBU; iv. [S]; v. CH_2N_2, Et_2O

Scheme 5.19

It seems the sequences illustrated in Scheme 5.20 should be a reasonable approach for the stereoselective synthesis of phosporothioate. Phosphorothioate **5-57** can be expected to be made stereoselectively as shown, and it then can react with diazomethane to form **5-58**, where the tetrazole nitrogen is blocked. It is reported[222] that the complete deprotection of tryptophan from N_1-phosphorylated tryptophan could be achieved with either sodium hydroxide or TBAF. Similarly the phosphorothioate should be obtained with sodium hydoxide treatment. Even more interesting is that the removal of tryptophan moiety by fluoride would result in the stereoselectiove synthesis of

[222] Guillaume, H. A.; Perich, J. W.; Johns, R. B.; Tregear, G. W. *J. Org. Chem.* **1989**, 54, 1664

phosphorofluoridite.[42] Unfortunately, lack of time did not allow this project to proceed any further.

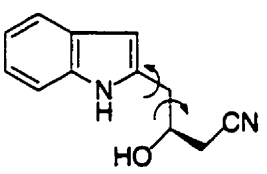


Scheme 5.20: A possible approach

Chapter 6. The Stereoselective Synthesis of Phosphorothioates Using Chiral Indolo-oxazaphosphorine Intermediates Derived from Tryptophans

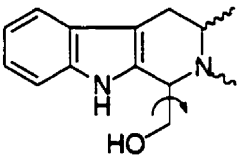
6.1. Structural Characteristics of the New Chiral Auxiliary

The imidazole or tetrazole moieties incorporated in the chiral auxiliaries tend to participate as discussed in Chapter 5. Should they be replaced by hydroxy groups, such participation would be avoided. As previously demonstrated in the indolo-oxazaphosphorine approach,[176,177] chiral auxiliary **26** led to the stereoselective synthesis of phosphorothioates in solution phase. When it was applied to the solid phase synthesis, a β-elimination caused rearrangement occurred to give a cyanoethyl phosphonate. We therefore decided to synthesize chiral auxiliary of type **A** which can be derived from tryptophan (Scheme 6.1). Instead of having two potential rotations as shown in **26**, structure **A** is more rigid, leaving only one rotation available. Eliminating the use of the cyanoethyl moiety in the chiral auxiliary would make the methodology more compatible with solid phase synthesis. When benzyl serves as a protecting group for N_b nitrogen of tryptophan, a Pictet-Spengler reaction tends to yield one single diastereomer. This would very well solve the chirality problem of the new chiral auxiliary, in contrast to the partial epimerization which happened in the process of making compound **26**. Furthermore, the chiral auxiliary can be derived from natural chiral tryptophans, making the method very attractive and inexpensive.



26

Previously used chiral auxiliary



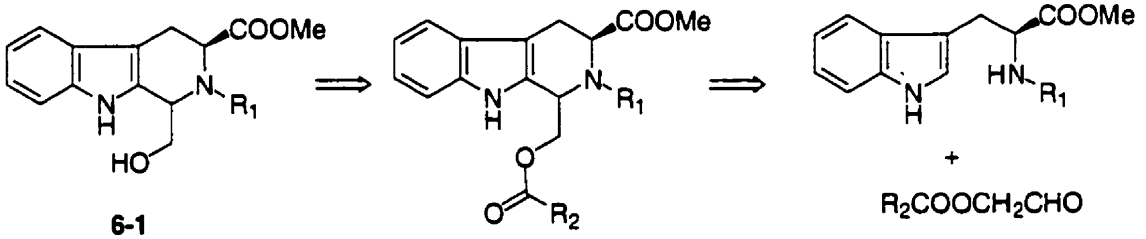
A

General structure of the new chiral auxiliary

Scheme 6.1: Structural characteristics of the new chiral auxiliary

6.2. Synthesis of the Chiral Auxiliary

6.2.1. Unexpected Lactone Formation



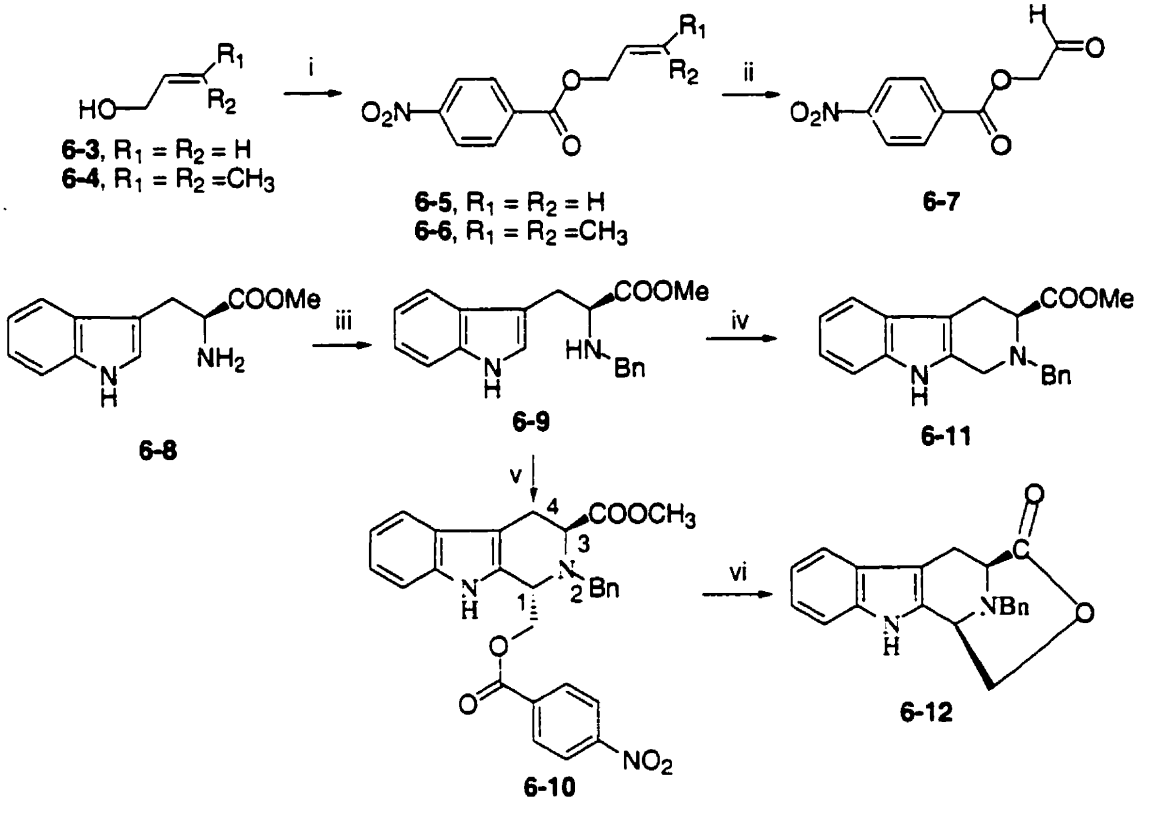
6-1 **6-2**

Scheme 6.2: Retrosynthetic Analysis

The retrosynthetic analysis leading to auxiliary **6-1** is shown in Scheme 6.2. Chiral auxiliary of type **6-1** can be obtained by hydrolysis of ester **6-2**, which can be prepared by the Pictet-Spengler condensation between an appropriate aldehyde and the N_b-protected tryptophan.

The synthesis started with the transformation of the allyl alcohol **6-3** to its *p*-nitrobenzoate ester **6-5** (Scheme 6.3). Ozonolysis of the latter then gave aldehyde **6-7**. Reductive amination of benzaldehyde with L-tryptophan methyl ester **6-8** yielded N_b-

benzyl tryptophan methylester **6-9**. Pictet-Spengler reaction between **6-7** and **6-9** in toluene without acid catalyst afforded the desired **6-10** accompanied by a side product **6-11** having a very close R_f value. We reasoned the formation of **6-11** was due to the Pictet-Spengler reaction between **6-9** and formaldehyde, which was formed by ozonolysis of allyl ester **6-5**. We then synthesized aldehyde **6-7** from dimethylallyl ester **6-6**, and then carried out the Pictet-Spengler reaction with **6-9**. A clean reaction occurred to give **6-10** as the only major product. Ester **6-10** was then hydrolyzed by reaction with sodium methoxide in methanol. To our big surprise, lactone **6-12** or its enantiomer was obtained as the product.



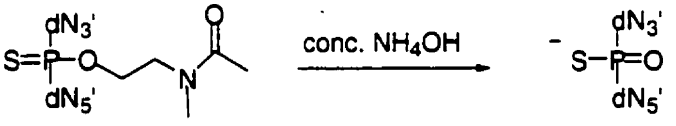
i. *p*-nitrobenzoyl chloride, pyridine, DMAP; ii. O_3, CH_2Cl_2, -78 °C;
iii. PhCHO, $NaCNBH_3$, MeOH, PH = 6; iv. HCHO, toluene, reflux, Dean-Stark trap;
v. **6-7**, toluene, reflux, Dean-Stark trap; vi. NaOMe, MeOH.

Scheme 6.3: Synthesis of the chiral auxiliary

As was shown by Cook et al.[213] the Pictet-Spengler reactions between N_b-benzyl methyl ester with aldehydes of large size led to stereospecific formation of the *trans* diastereomer. Since our product was a single isomer, it follows that the epimerization occurs readily at C1 or C3. These facile epimerizations are precedented.[212] We therefore thought of synthesizing the chiral auxiliary with an amide group.

6.2.2. Neighboring Amide Group Participation

It was reported[223,224] by Iyer et al. that the reaction of a 2-acetamidoethylphosphate triester with aqueous ammonia gave the corresponding diester as illustrated in Scheme 6.4.

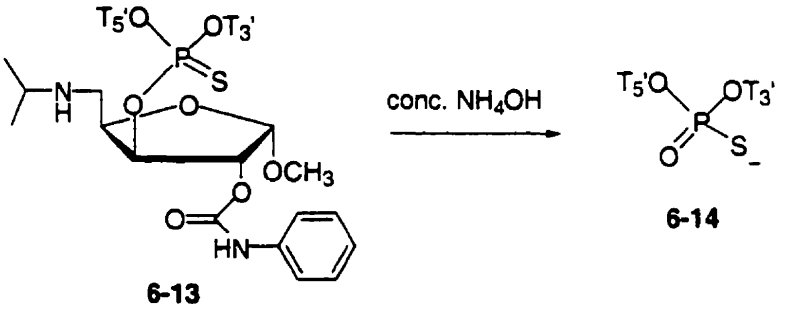


Scheme 6.4: The neighboring group participation of acetamidoethyl group

Marsault and Just[225] also synthesized oxazaphosphorinane **6-13** possessing a participating group. Treatment with concentrated ammonia quickly resulted in the elimination of diester **6-14** from triester **6-13** (Scheme 6.5).

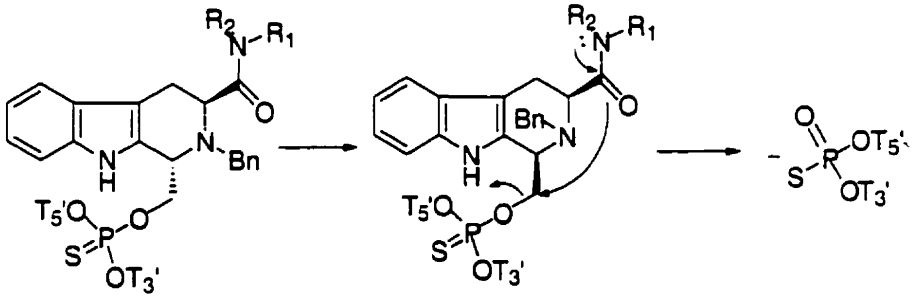
---

[223] Iyer, R. P.; Yu, D.; Devlin, T.; Ho, N.-H. and Agrawal, S. *J. Org. Chem.* **1995**, 60, 5388
[224] Iyer, R. P.; Yu, D.; Ho, N.-H.; Tan, W.; Agrawal, S. *Tetrahedron: Asymmetry* **1995**, 6, 1051
[225] Marsault, E. and Just, G. *Tetrahedron* **1997**, 53, 16945



Scheme 6.5: Oxazaphosphorinane with a participating group

We therefore considered synthesizing a chiral auxiliary with an amide group as depicted in Scheme 6.6. Its removal by neighboring group participation can be expected at the end of the synthesis, should there be a facile epimerization during the removal.

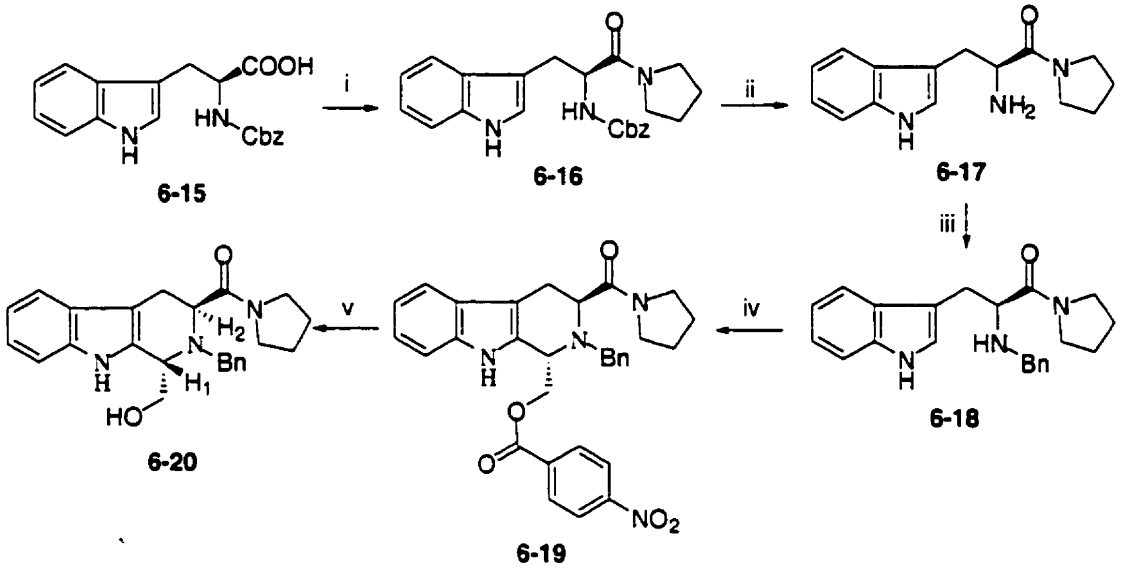


Scheme 6.6: Neighboring group participation in the chiral auxiliary

6.2.3. Synthesis of Chiral Auxiliary with Neighboring Amide Group

Chiral auxiliary **6-20** possessing a neighboring pyrrolidine amide group was easily prepared as outlined in Scheme 6.7. Commercially available N_b-(benzyloxycarbonyl)-L-tryptophan **6-15** was coupled with pyrrolidine to yield amide **6-16**. The benzyloxycarbonyl (cbz) protecting group was then removed to give **6-17**, which upon reductive amination with benzaldehyde was transformed into **6-18**. Pictet-Spengler

condensation of **6-18** with aldehyde **6-7** afforded **6-19** as a single diastereomer, which was then hydrolyzed to furnish **6-20** as one isomer. The stereochemistry of **6-20** can not be established by 2D-NOESY. There were no cross peaks observed between the H_1 and H_2 protons in the NOESY spectra of **6-20**.



i. pyrrolidine, Et_3N, HBTU, CH_3CN, RT; ii. H_2, Pd/C, ethyl acetate/H_2O/AcOH; iii. PhCHO, $NaCNBH_3$, MeOH, PH = 6; iv. **6-7**, toluene, reflux, Dean-Stark trap; v. NaOH, H_2O/THF, 55 °C

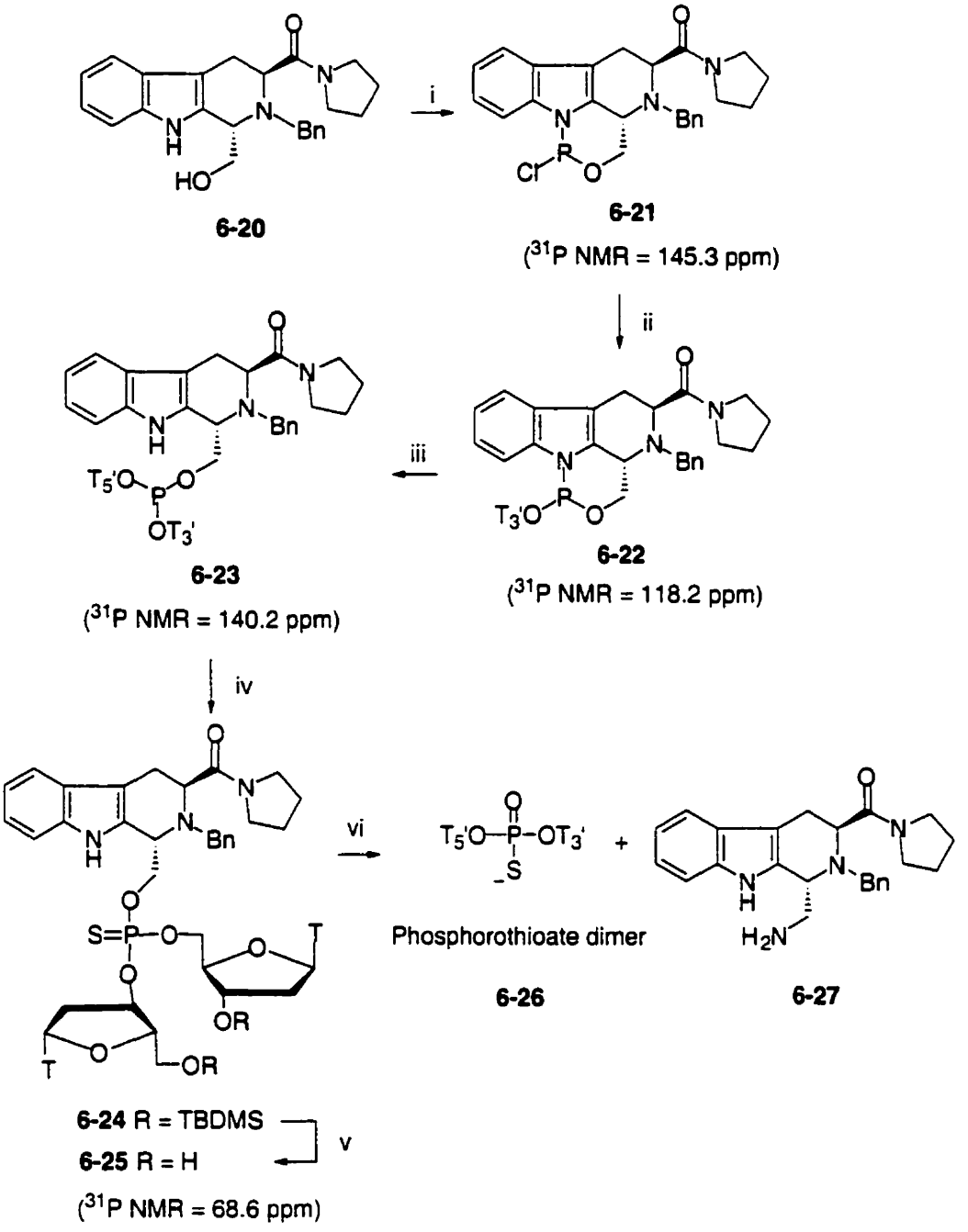
Scheme 6.7: Synthesis of the chiral auxiliary possessing a neighboring amide group

6.3. Stereoselective Synthesis of Phosphorothioates

The application of chiral auxiliary **6-20** to the stereoselective synthesis of phosphorothioates is outlined in Scheme 6.8. The solution of **6-20** and triethylamine in dichloromethane was added slowly to phosphorus trichloride at 0 °C. Initially several ^{31}P NMR resonances were observed. After heating at 50 °C for one day to equilibrate, one single resonance at around 145 ppm was observed, corresponding to **6-21**. When intermediate **6-21** was further reacted with T_3·OH in the presence of triethylamine in

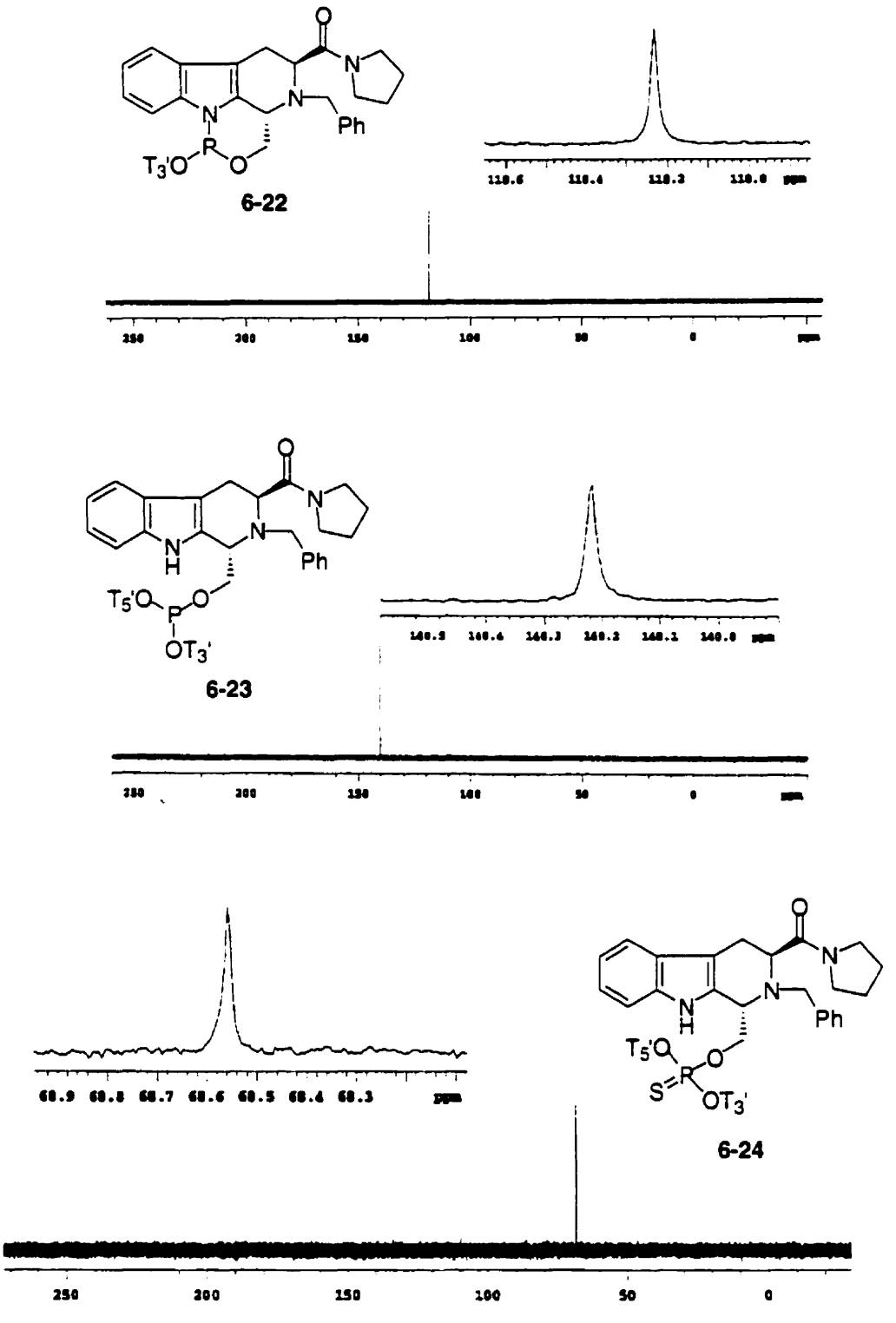
dichloromethane, the reaction proceeded smoothly to yield a single major isomer of phosphoramidite **6-22** with ^{31}P NMR at 118.2 ppm. Cyclic phosphoramidite **6-22** was purified by silica gel column chromatography. When pure **6-22** was treated with T_5-OH in presence of DBU, the displacement was completed within half an hour at room temperature, as established by the formation of a major, characteristic ^{31}P NMR peak at 140 ppm corresponding to phosphite triester **6-23**. DBU was removed by filtration through a short column, and sulfurization with Beaucage's reagent afforded phosphorothioate **6-24** with a major ^{31}P NMR resonance at around 68 ppm. Removal of silyl protecting groups gave dithymidine phosphorothioate **6-25**. The ^{31}P NMR spectra of some intermediates are shown in Scheme 6.9.

We next attempted to remove the chiral auxiliary. The solution of **6-25** in water/THF was first heated at 55 °C. No reaction occurred after 1 day. We then treated **6-25** with concentrated ammonia/ethanol (3:1) at 55 °C for 16 hours. To our delight, phosphorothioates **6-26** ware obtained, as indicated by ^{31}P NMR the presence of two peaks at 56.1 ppm and 56.0 ppm in a ratio of 1 to 40. The configurations of minor and major isomers were assigned as S_P and R_P, respectively, based on their ^{31}P NMR chemical shifts and HPLC retention times. The chiral auxiliary we recovered had a structure **6-27**, which means direct attack from ammonia was responsible for the removal of the chiral auxiliary. Should the chiral auxiliary epimerize to give a *cis* configuration, neighboring group participation would be expected to yield lactone **6-12** or species **6-28** after the deprotection (Scheme 6.10).

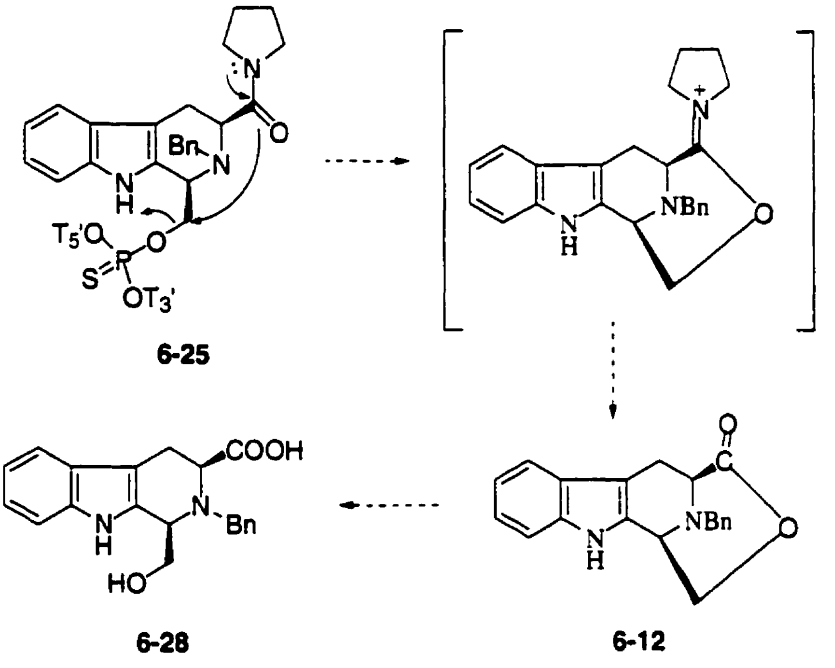


i. PCl_3, Et_3N, CH_2Cl_2; ii. T_3-OH, Et_3N, CH_2Cl_2; iii. T_5-OH,DBU, THF;
iv. Beaucage's reagent; v. TBAF; vi. conc. ammonia/EtOH, 55 °C.

Scheme 6.8: Stereoselective synthesis of phosphorothioates

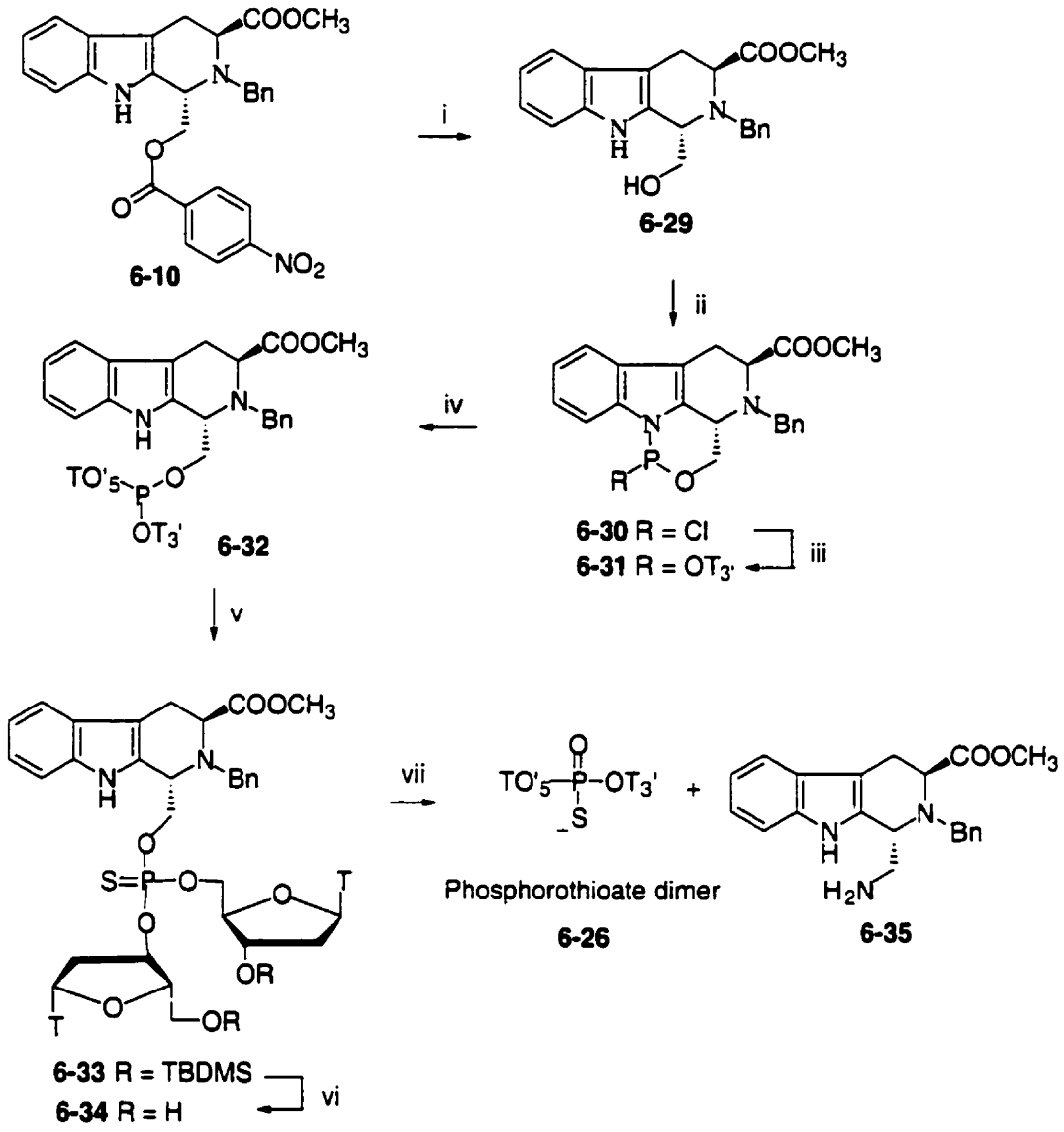


Scheme 6.9: Selected ^{31}P NMR spectra of some intermediates



Scheme 6.10

If direct nucleophilic attack from ammonia was responsible for the removal of the chiral auxiliary, then a chiral auxiliary without neighboring amide group should also be removable under the same conditions. Scheme 6.11 shows the synthesis of methyl ester chiral auxiliary and its use for the stereoselective synthesis of dithymidine phosphorothioate. The solution of **6-10** in isopropylamine was heated to 80 °C in a sealed vessel for 10 hours to furnish chiral auxiliary **6-29**. Through a similar sequence of reactions as previously described, phosphorothioate **6-34** was obtained. It was then heated in concentrated ammonia/ethanol (3:1) at 55 °C to effect the removal of the chiral auxiliary. Dithymidine phosphorothioate **6-26** and amino chiral auxiliary **6-35** were obtained as expected. This result confirmed that the direct nucleophilic attack by ammonia removed the chiral auxiliary.

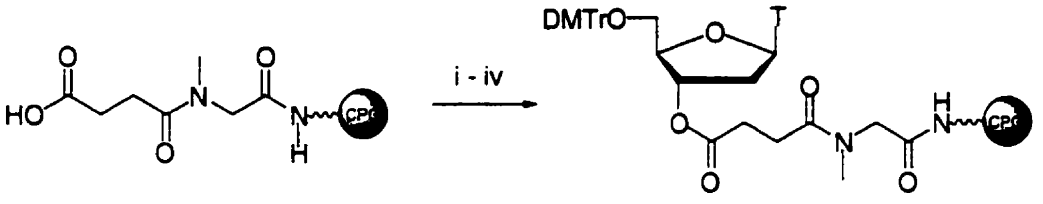


i. *i*-$PrNH_2$, 80 °C; ii. PCl_3, Et_3N, CH_2Cl_2; iii. T_3OH, Et_3N, CH_2Cl_2; iv. T_5OH, DBU, THF; v. Beaucage's reagent; vi. TBAF; vii. conc. ammonia/ETOH,55 °C

Scheme 6.11: Stereoselective synthesis of phosphorothioates from methyl ester chiral auxiliary

6.4. Solid Phase Synthesis of R_P- and S_P– dithymidine Phosphorothioates

To demonstrate our methodology is compatible with solid phase synthesis of oligonucleotides, we then synthesized R_P- and S_P–dithymidine phosphorothioates on the solid phase.



i. 4-DMAP, DEC, pyridine; ii. pentachlorophenol; iii. piperidine;
iv. Ac_2O, DMAP, 2,4,6-trimethylpyridine, THF

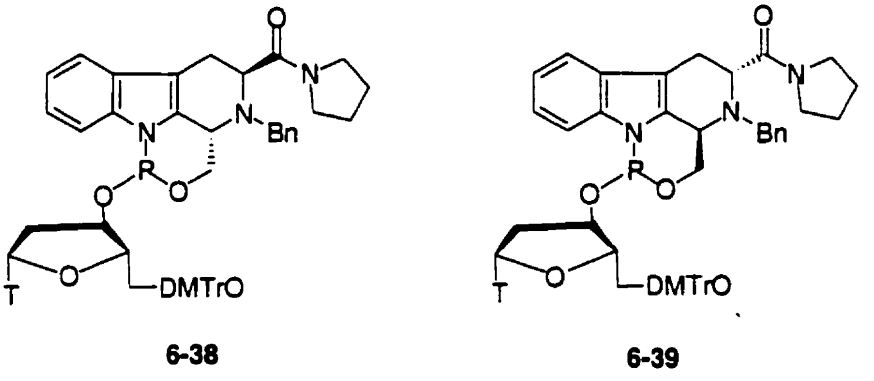
Scheme 6.12: Load of 5'-O-DMTr-thymidine to the solid support

In our methodology, the displacement of the indole moiety in indolo-oxazaphosphorine **6-22** with T_3·OH was activated by DBU. It was reported[226,227] that DBU partly cleaves the standard linker (-$COCH_2CH_2CO$-LCA-) and releases the nucleotide from the solid support. Therefore, 5'-O-DMTr-thymidine was immobilized on controlled pore glass (CPG) via a DBU-resistant sarcosinyl-succinolyl linker (-CO $CH_2CH_2CON(Me)$-CH_2CO-LCA-). The loading was done according to the literature procedure.[228] As shown in Scheme 6.12, a mixture of 5'-O-DMTr-thymidine, CPG with a sarcosinyl-succinoyl linker **6-36**, 4-DMAP, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (DEC) and anhydrous pyridine were shaken at room temperature for

[226] Brown, T.; Pritchard, C. E.; Turner, G.; Salisbury, S. A. *J. Chem. Soc. Chem. Commun.* **1989**, 891
[227] Lehman, C.; Xu, Y.; Christoloulos, C.; Tan, Z. K.; Gait, M. J. *Nucleic Acid. Res.* **1989**, 17, 2379

24 hours to furnish nucleoside coupled CPG **6-37**. Following the coupling, the residual carboxyl groups were converted to amides by addition first of pentachlorophenol/DEC and then piperidine. Finally, the resulting CPG was treated with acetic anhydride and 4-DMAP to esterify additional hydroxy groups.

The synthesis of the monomers were carried out using similar procedures as for their silyloxy analog **6-22**. Cyclic phosphoramidites **6-38** and **6-39** were derived from L-tryptophan and D-tryptophan, respectively (Scheme 6.13).



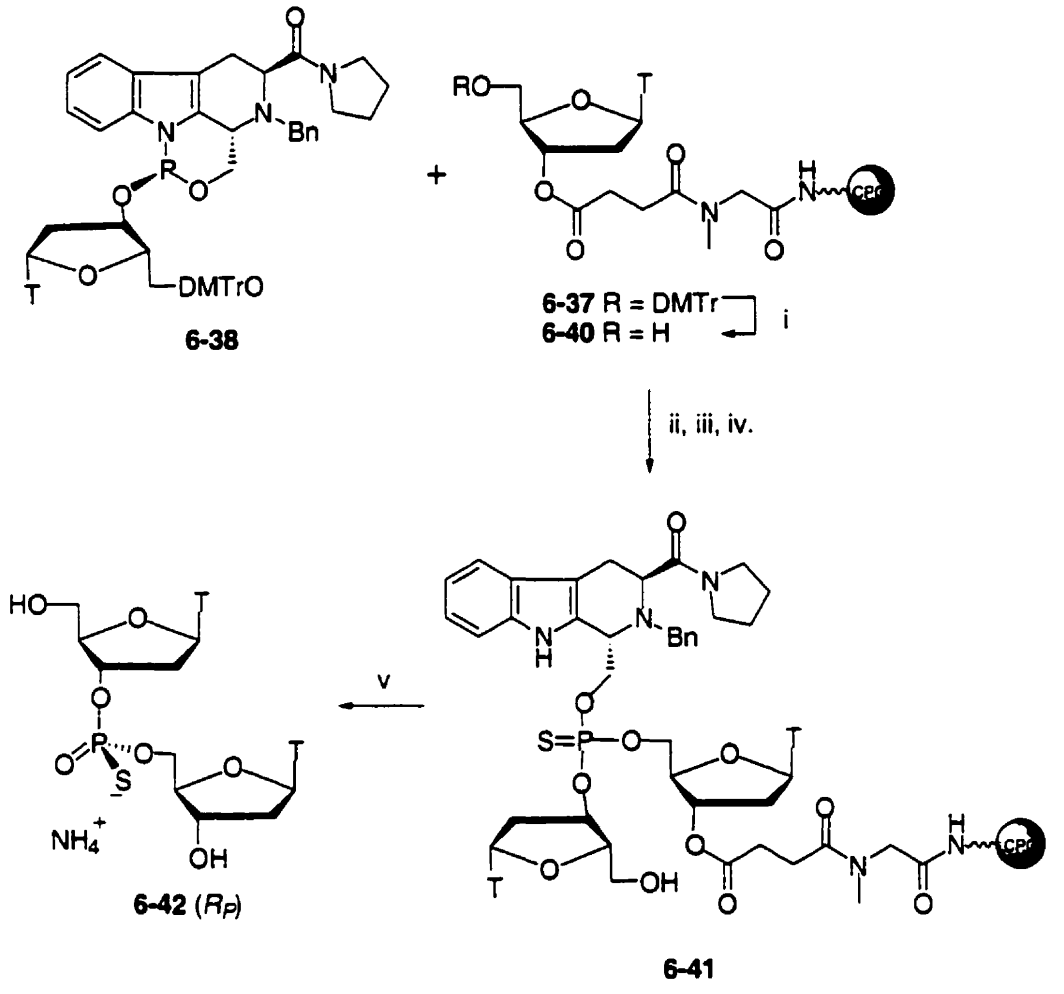
6-38 **6-39**

Scheme 6.13: Monomers for solid phase synthesis

The solid phase synthesis of dithymidine phosphorothioates were performed manually (Scheme 6.14). The immobilized thymidine **6-37** in a sintered glass funnel was treated with the trichloroacetic acid detritylation solution to afford **6-40**. It was then reacted with excess DBU first, followed by the addition of monomer **6-38**. After 30 minutes at room temperature, the solid support was washed with acetonitrile and sulfurized with Beaucage's reagent, followed by detritylation to furnish phosphorothioate

[226] Damha, M. J.; Giannaris, P. A.; Zabarylo, S. U.; *Nucleic Acid. Res.* **1990**, 18, 3813

6-41. The solid support was heated in concentrated ammonia at 55 °C to cleave dimer and remove the chiral auxiliary to furnish R_P–dithymidine phosphorothioate **6-42**.

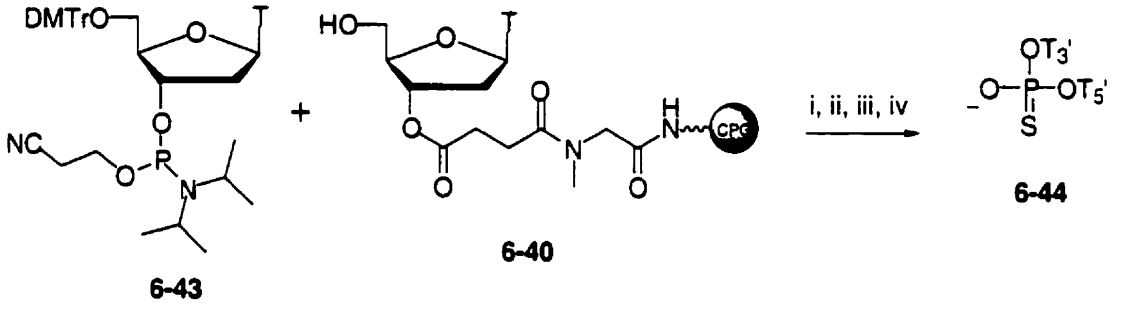


i. Cl_3CCOOH; ii. DBU, then **6-38**; iii. [S]; iv. Cl_3CCOOH; v. conc. ammonia/EtOH, 55 °C

Scheme 6.14: Solid phase synthesis of R_P- dimer

In order to assign the configuration of **6-42**, we synthesized dithymidine phosphorothioates using the standard, non-stereoselective method. Cyanoethyl phosphoramidite **6-43** was coupled with immobilized thymidine in the presence of

tetrazole. The phosphorothioates **6-44** obtained in the end consisted of a mixture of R_P and S_P isomers (Scheme 6.15). This mixture of isomers was used as a control.



i. tetrazole, CH_3CN; ii. [S]; iii. Cl_3COOH; iv. NH_4OH, 55 °C

Scheme 6.15: Non stereoselective synthesis of phosphorothioates

The absolute configuration of **6-42** was established by comparison of reverse phase (RP)-HPLC spectra of **6-42** and **6-44**. It was documented in the literature[229,230] that the R_P isomer of the dinucleotide eluted faster than S_P isomer in RP-HPLC. The HPLC spectra are shown in Scheme 6.16, the configuration of **6-42** was accordingly assigned as R_P. The identity of **6-42** was also proved by HPLC-MS, which showed the mass of dithymidine phosphorothioate anion at 561.

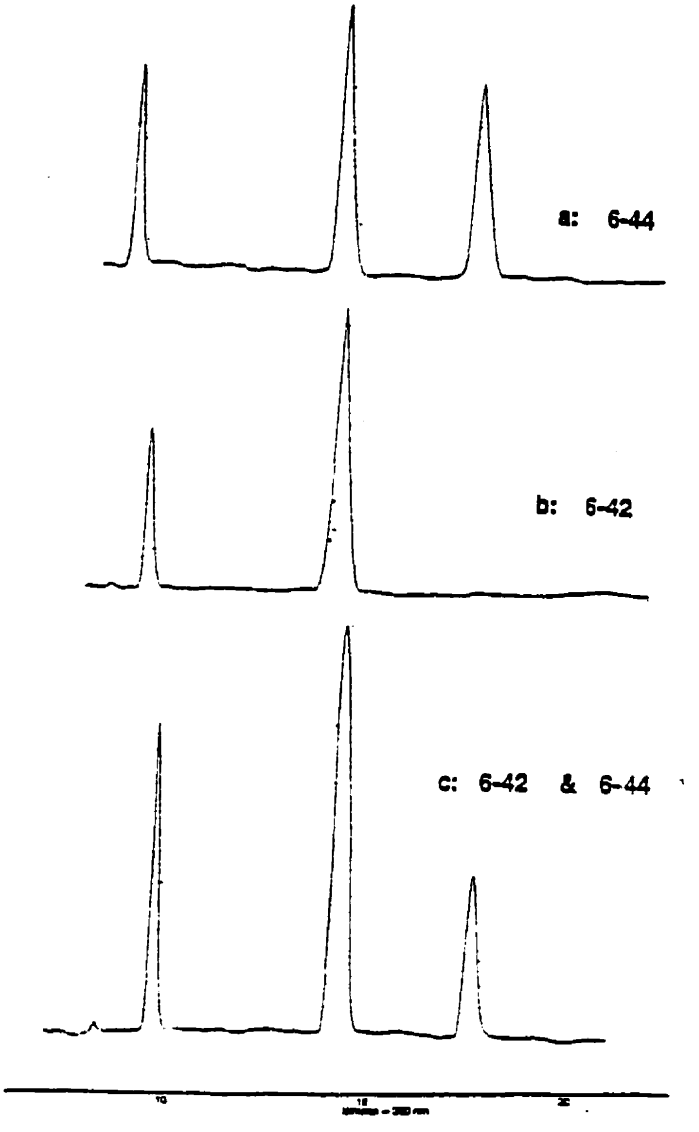
Similarly, **6-39** led to the formation of S_P dimer which was also confirmed by HPLC comparison and HPLC-MS analysis.

---

[229] Stec, W. J.; Zon, G. and Uznanski, B. *J. Chromtogr.* **1985**, 326, 263

[230] Kanehara, H.; Wada, T.; Mizugachi, M. and Makino, K. *Nudleosides and Nucleotides* **1996**, 15, 1169

In summary, chiral indolo-oxazaphosphorine auxiliaries can be easily prepared form L- and D-tryptophans. While applied in both solution and solid phase synthesis of dithymidine phosphorothioates, the L-tryptophan derived chiral auxiliary led to the formation of R_P isomer, and D-tryptophan derived precursor led to the formation of S_P isomer.

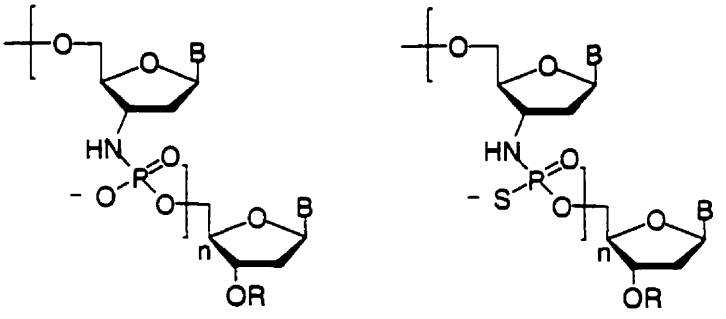


Scheme 6.16: HPLC spectra of 6-42 and 6-44 *

* HPLC conditions: Phenomenex C8 column; solvent A: water; solvent B: acetonitrile; flow rate: 1.5 ml/min; 3% B increased to 7% for the first 30 minutes, then to 40% during the next 20 minutes.

6.5. The Stereoselective Synthesis of N3'-P5' Thiophosphoramidates

Among novel oligonucleotide analogs, oligonucleotide N3'-P5' phosphoramidate (**A**) (Scheme 6.17) is a promising modification,[231,232] which showed higher stability and better binding affinity towards DNA or RNA strands in comparison with natural phosphodiesters. Moreover, oligonucleotide N3'-P5' phosphoramidates were more potent antisense agents than phosphorothioate derivatives both *in vitro* and *in vivo*.[233] Recently, N3'-P5' thiophosphoramidates (**B**) were also developed and found to retain high RNA binding affinity of the parent oligonucleotide N3'-P5' phosphoramidates and exhibit a much higher acid stability. Introducing a sulfur atom would apparently create a new chiral center at the phosphorus. The protocol described by Gryaznov et al.[234] was non-stereoselective, we therefore tried to apply our chiral auxiliary to the stereoselective synthesis of N3'-P5' thiophosphoramidate to demonstrate the generality of our methodology.

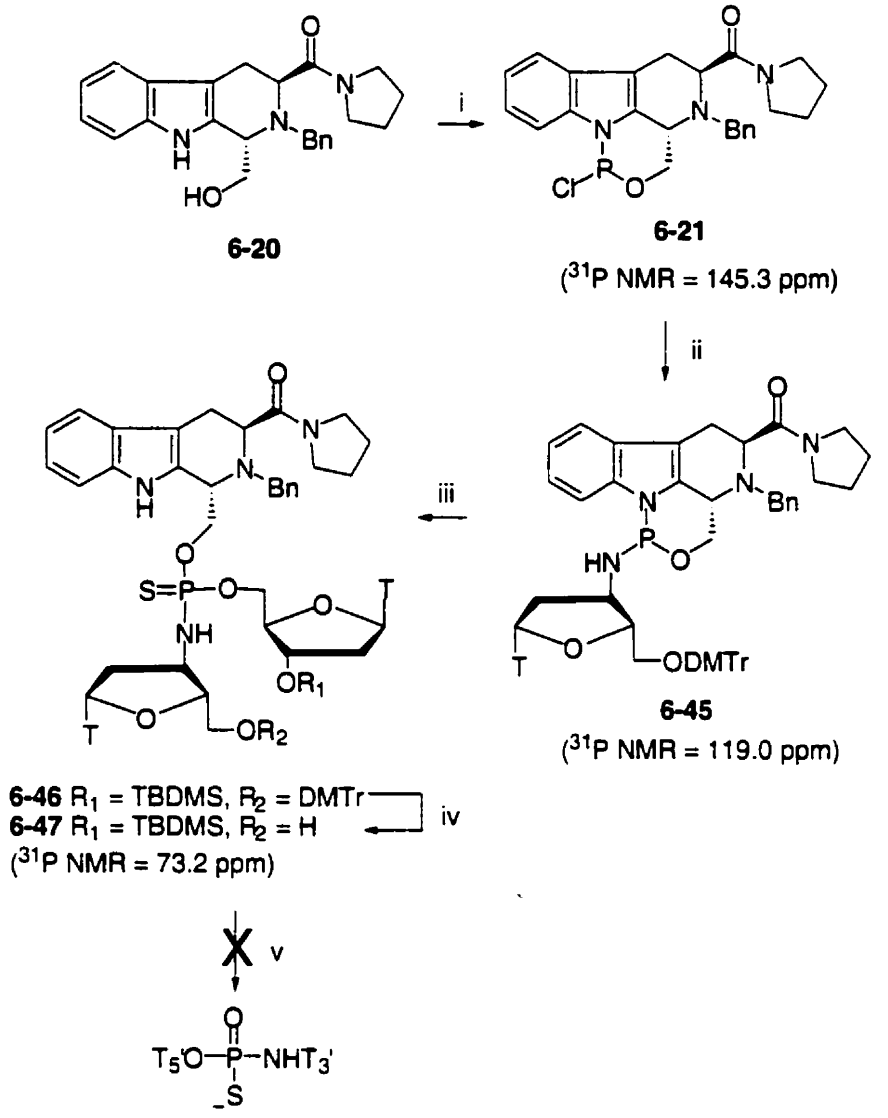


Scheme 6.17: N3'-P5' phosphoramidate

[231] Gryaznov, S.; Lloyd, D.; Chen, J.; Schultz, R.; DeDinonisio, L.; Ratmeyer, L.; Wilson, W. D. *Proc. Natl. Acad. Sci. USA* **1995**, 92, 5798

[232] Gryaznov, S.; Chen, J. *J. Am. Chem. Soc.* **1994**, 116, 3143

[233] Skorski, T.; Perrotti, D.; Nieborowska-Skorska, M.; Gryaznov, S.; Calabretta, B. *Proc. Natl. Acad. Sci. USA* **1997**, 94, 3966



i. PCl_3, Et_3N, CH_2Cl_2; ii. T_3NH_2, Et_3N, CH_2Cl_2; iii. T_5OH,DBU, THF, 55 °C then Beaucage's reagent; iv. AcOH; v. conc. ammonia/EtOH, or $(CH_3)_2NH$, 55 °C

Scheme 6.18: Stereoselective synthesis of N3'-P5' thiophoshoramidate

The stereoselective synthesis of N3'-P5' thiophosphoramidate is depicted in Scheme 6.18. Reaction of chiral auxiliary **6-20** with phosphorus trichloride yielded phosphorus monochloride **6-21** with a single ^{31}P NMR resonance at 145 ppm. Subsequent

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[234] Pongracz, K.; Gryaznov, S. *Tetrahedron Lett.* **1999**, 40, 7661

reaction with 5'-O-DMTr-3'-NH_2-thymidine gave cyclic phosphoramidite **6-45** as a single diastereomer after flash chromatography. In contrast to the fast displacement of indole moiety in the indolo-oxazaphosphorine intermediate, the reaction of **6-45** and T_5-OH in the presence of DBU went very slowly. The reaction was not completed even after 10 hours at room temperature. We were able to drive the reaction to completion when the reaction was run at 55 °C for 2 hours. Subsequent sulfurization, followed by the removal of the trityl group furnished thiophosphoramidate **6-47** as a single diastereomer with a ^{31}P NMR resonance at 73 ppm. The chiral auxiliary could not be removed by treatment with concentrated ammonia or 40% aqueous dimethyl amine at 55 °C. Unfortunately, **6-47** got hydrolyzed before we tested the feasibility of chiral auxiliary removal by sodium thiophenoxide. We did not proceed any further on this project due to lack of time.

Contribution to the Knowledge

Different catalysts 2-iodo, 2-nitro and 2-mesitylene-4,5-dicyanoimidazoles **2-5**, **2-8** and **2-10** were synthesized.

The novel xylose derived chiral auxiliaries **3-16** and **3-32** with tertiary carbons at 3-position were synthesized.

Both the single protonation mechanism and the double inversion mechanism are the possible pathways in the coupling reactions of phosphoramidites **2-15**, **3-18** or **3-34**.

The novel, facile removal of the allyl protecting group for the internucleotide linkage of phosphorothioate and phosphate was achieved by treatment with nucleophiles. This protocol can be used for the routine solid phase synthesis of oligonucleotide phosphorothioate and phosphate.

A new type of chiral auxiliary **5-20** incorporating indole and imidazole was synthesized. It led to the stereoselective synthesis of a novel indolophosphorothioate **5-37** with 90% de and the formation of an alkylphosphinate analog **5-41** as a single diastereomer.

A novel indolo-oxazaphosphorine **6-20** was synthesized. A methodology for the stereoselective synthesis of phosphorothioate with ≥95% de was developed by the use of **6-20** as the chiral precursor. Both R_P and S_P diastereomers of dithymidine phosphorothioates can be obtained by using chiral auxiliaries derived from either L- or D-tryptophan. The methodology was applied successfully to the solid phase synthesis of dithymidine phosphorothioate.

Chapter 7. Experimental Section

7.1. General Methods

^{1}H NMR spectra were recorded on a Varian XL200, Unity 500 or a Jeol Eclipse 270 spectrometer. ^{13}C NMR were recorded on a Unity 500 spectrometer. Chemical shifts are always given in the δ scale in parts per million (ppm). The assignments of proton spectra are based on COSY experiments, the assignments of carbon spectra were determined with the help of DEPT or HMQC pulse sequences when appropriate. The ^{1}H NMR spectra were referenced with respect to the residual signals of deuterated chloroform (δ = 7.24 ppm), methanol (δ = 3.30 ppm), acetone (δ = 2.04 ppm). The ^{13}C NMR spectra were referenced with respect to the signals of deuterated chloroform (δ = 77.0 ppm), methanol (δ = 49.0 ppm), acetone (δ = 205.4 ppm). The ^{31}P NMR spectra were referenced externally with an 85 % solution of phosphoric acid (δ = 0 ppm). The multiplicities were given according to the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), h (heptet), m (multiplet), b (broad singlet).

Melting points were determined using a Gallenkamp MF-370 electrothermal apparatus and are uncorrected.

Mass spectra were recorded on MS25RFA and ZAB 2F HS mass spectrometers.

Tetrahydofuran and diethyl ether were freshly distilled from sodium benzophenone ketyl, dichloromethane and chloroform from phosphorus pentoxide, methanol from magnesium, triethylamine, diisopropylamine and acetonitrile from calcium hydride, pyridine from barium oxide, benzene from sodium metal. Anhydrous

DMF was purchased from Aldrich Chemical Company Inc. in sure-seal bottles and used with no further drying.

Phosphorus trichloride was first degassed by refluxing for 2 hours under argon followed by fractional distillation and was stored under argon. All other chemicals were purchased from Aldrich Chemical Inc., Fluka Chemicals when appropriate and were used without further purification. Tetrazole and Beaucage's reagent were gifts from Isis Pharmaceuticals (Carlsbad, CA).

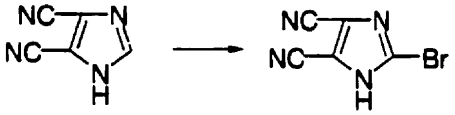
Thin layer chromatography was performed using Kieselgel $60F_{254}$ aluminum-backed plates (0.2 mm thickness) and visualized when appropriate by exposure under a U.V. light and by dipping into a solution of ammonium molybdate (2.5 g) and ceric sulfate (1.0 g) in 10 % v/v aqueous sulfuric acid followed by heating. Flash chromatography was performed using the method described by Still et al.[235], on silica gel Kieselgel 60 (Merck, 230-400 mesh).

Glassware was heated in an oven at 140 °C overnight, then cooled in a desiccator containing drierite or granular phosphorus pentoxide.

[235] Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, 43, 2923

7.2. Experimentals for Chapter 2

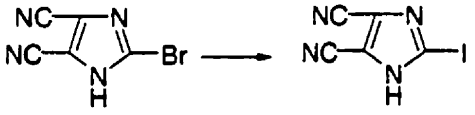
2-Bromo-4,5-dicyanoimidazole 2-4



2-4

To a solution of 4,5-dicyanoimidazole (1.18 g, 10 mmol) in 0.1 M sodium hydroxide (25 ml) was added bromine (1.8 ml, 35 mmol). The mixture was stirred overnight at room temperature and then acidified with 1N hydrochloric acid. The solid was filtered, rinsed with water and recrystallized from water to give white crystalline 2-bromo-4,5-dicyanoimidazole (1.5 g, 76%), m.p. 146-148 °C (literature[181]: 141 °C); MS (EI) m/z 198 ((M+2), 96%), 196 (M^+, 100%).

2-Iodo-4,5-dicyanoimidazole 2-5

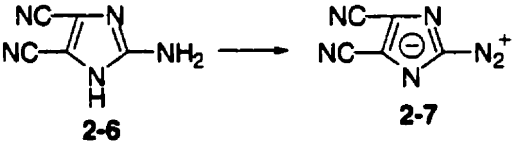


2-4 **2-5**

A mixture of 2-bromo-4,5-dicyanoimidazole **2-4** (0.20 g, 1 mmol), potassium iodide (1.66 g, 10 mmol), copper(I) iodide (0.95 g, 5 mmol) in hexamethylphosphorus triamide (HMPA) (5 ml) was heated to 120 °C for 2 days. The reaction was quenched by the addition of 2 N hydrochloric acid solution (20 ml) followed by ethyl acetate (20 ml). The organic layer was washed successively with aqueous sodium carbonate, 2N hydrochloric acid and water. and dried over magnesium sulfate. The solvent was removed *in vacuo*. The desired product was obtained as a yellow solid (0.15 g, 61%).

^{13}C NMR (75.3 MHz, CD_3COCD_3) δ 130.6, 124.3, 116.5; MS (EI) m/z 244 ($(M+1)^-$, 29.9%).

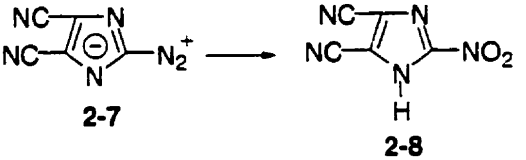
2-Diazo-4,5-dicyanoimidazole 2-7



2-6 **2-7**

To a suspension of 2-amino-4,5-dicyanoimidazole (1.33 g, 10 mmol) in water (30 ml) at room temperature was added concentrated hydrochloric acid (7.5 ml). The imidazole was dissolved. The solution was cooled down by ice bath and a solution of sodium nitrite (1 g, 14 mmol) in water (3 ml) was added dropwise while keeping the temperature of the solution below 0 °C. The precipitate was formed quantitatively. The mixture was stirred at 0 °C for a few more minutes and the solid was collected by filtration. Wet **2-7** was transferred and dried under vacuum. White solid was obtained as the desired product (1.37 g, 95%). The salt was used directly for the following reactions without further purification.

2-Nitro-4,5-dicyanoimidazole 2-8



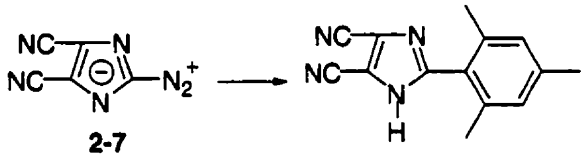
2-7 **2-8**

To **2-7** (0.14 g, 1 mmol) obtained previously was added sodium nitrite (0.07 g, 1 mmol) in water at room temperature. The mixture was stirred until TLC showed the disappearance of starting material. The product was taken up in ethyl acetate, washed and

dried. Purification by flash column chromatography afforded the desired product as a yellow solid (0.19 g, 80%).

MS (EI) m/z 162 (M-1).

2-Mesitylene-4,5-dicyanoimidazole 2-10

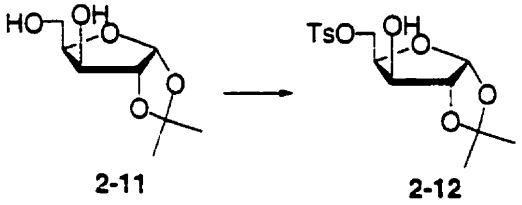


2-7 **2-10**

Diazonium salt **2-7** (0.14 g, 1 mmol) was dissolved in mesitylene (10 ml). The mixture was brought to reflux overnight. After cooling, yellow solid precipitated out. Recrystallization from ethyl acetate/hexanes afforded the desired product as yellow needle crystals (0.16 g, 70%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 6.78 (s, 1H, aromatic H), 6.65 (s, 1H, aromatic H), 1.91–2.19 (3 s's, 9H, 3xCH_3); ^{13}C NMR (75.3 MHz, $CDCl_3$) δ 138.1, 128.5, 126.7, 125.4, 124.4, 110.8, 63.9, 20.4, 19.1; MS (EI) 236 (100%, M-1), 221 (M-1-CH_3, 27.9%), 206 (M-1-2xCH_3, 8.2%).

1,2-Di-O-isopropylidene-5-O-tosyl-D-xylofuranose 2-12



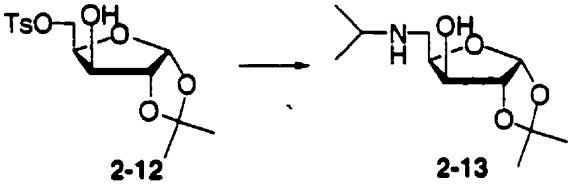
2-11 **2-12**

p–Toluenesulfonyl chloride (0.21 g, 1.1 mmol) was added to a solution of 1,2-di-O-isopropylidene-D-xylofuranose **2-11** (0.19 g, 1 mmol) in pyridine (5 ml) at 0 °C under

argon. The resulting mixture was stirred at room temperature for 3 hours. Water was then added to quench the reaction and the solvent was removed under high vacuum, coevaporated with toluene twice. The residue was taken up in ethyl acetate, washed with water and brine, and dried over magnesium sulfate. Recrystallization from THF/ether furnished **2-12** (0.31 g, 90%) as a white solid.

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.26-7.82 (m, 4H, Ph), 5.88 (d, 1H, H-1, J_{1-2} = 3.6 Hz), 4.51 (d, 1H, H-2, J_{1-2} = 3.6 Hz), 4.28-4.35 (m, 3H, H-5, H-3), 4.15 (m, 1H, H-4), 2.45 (s, 3H, OCH_3), 2.22 (d, 1H, OH, J = 4.8 Hz), 1.46 (s, 3H, CCH_3), 1.30 (s, 3H, CCH_3); MS (CI) m/z 345 $(M+1)^+$.

1,2-Di-O-isopropylidene-5-deoxy-5-isopropylamino-D-xylofuranose 2-13



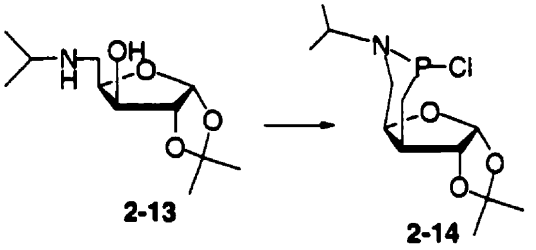
2-12 **2-13**

1,2-Di-O-isopropylidene-5-O-tosyl-D-xylofuranose **2-12** (0.33 g, 1 mmol) was dissolved in isopropylamine (4 ml) and heated to 55 °C for 36 hours in a pressure bottle. The solvent was removed *in vacuo*. The residue was taken up in dichloromethane, washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over magnesium sulfate. Purification by flash column chromatography afforded the desired product **2-13** as a white solid (0.18 g, 79%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 8.0 (br, 1H, NH), 5.91 (d, 1H, H-1, J_{1-2} = 3.7 Hz), 4.44 (d, 1H, H-2, J_{1-2} = 3.7 Hz), 4.18-4.25 (m, 2H, H-3, H-4), 2.90-3.39 (m, 2H, H-5), 2.66-2.79 (1H, septet, NCH), 1.44 (s, 3H, CCH_3), 1.28 (s, 3H, CCH_3), 1.03 (d, 6H, $CH(CH_3)_2$, J =

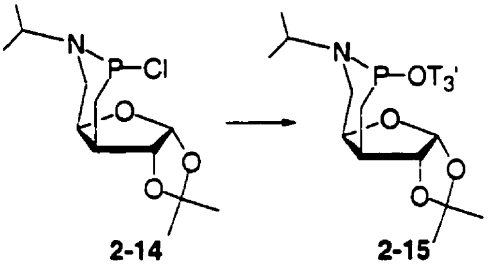
6.4 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.43, 105.12, 86.14, 78.23, 76.91, 48.84, 45.90, 26.81, 26.17, 22.64, 22.34; MS (CI) m/z 232 ($(M+1)^+$, 100%).

Cyclic Phosphorochloridite 2-14



To a solution of freshly distilled phosphorus trichloride (96 μl, 0.11 mmol) in dry dichloromethane (0.2 ml) in a NMR tube at 0 °C was added the solution of 1,2-di-O-isopropylidene-5-deoxy-5-isopropylamino-D-xylofuranose **2-13** (23 mg, 0.10 mmol) in dichloromethane (0.45 ml) containing triethylamine (30.7 μl, 0.22 mmol). The NMR tube was then sealed and the reaction was followed by ^{31}P NMR. When a single peak at around 148 ppm was observed, the reaction proceeded to next step without further purification.

Oxazaphosphorine 2-15

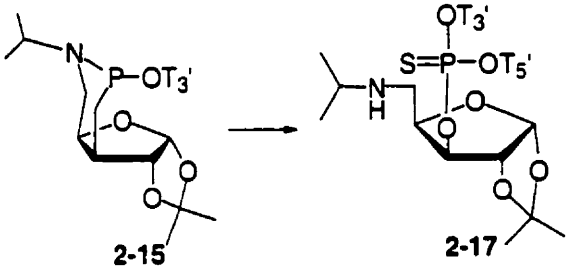


To **2-14** in a NMR tube was added a solution of T_3·OH (39 mg, 0.11 mmol) in dichloromethane (0.5 ml) containing triethylamine (15.3 μl, 0.11 mmol). The NMR tube

was sealed and heated at 50 °C overnight. The reaction mixture was diluted with dichloromethane, washed with saturated sodium bicarbonate and water, and dried over magnesium sulfate. Flash column chromatographic purification using triethylamine as part of the eluant afforded the desired product as a white solid (50 mg, 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (s, 1H, H-6), 6.32-6.33 (dd, 1H, H-1', J = 8.3 Hz, 5.4 Hz), 5.91 (d, 1H, H-1'', J = 3.5 Hz), 4.56 (m, 1H, H-3'), 4.49 (d, 1H, H-2'', J = 4.0 Hz), 4.34 (s, 1H, H-3''), 4.16 (d, 1H, H-4'', J = 2.0 Hz), 4.06 (m, 1H, H-4'), 3.77-3.89 (2H, H-5'), 3.39-3.47 (m, 2H, H-5'', NCH), 3.01-3.01 (m, 1H, H-5''), 2.35-2.38 (m, 1H, H-2'), 2.05-2.10 (m, 1H, H-2'), 1.89 (s, 3H, CH_3C=C), 1.47 (s, 3H, C**H$_3$**CCH_3), 1.29 (s, 3H, CH_3CC**H$_3$**), 1.09-1.12 (m, 6H, (C**H$_3$**$)_2CHN$, 0.90 (s, 9H, $SiC(CH_3)_3$), 0.10 (s, 6H, $Si(CH_3)_2$; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 130.2 ppm; ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.66, 150.27, 135.21, 111.71, 110.96, 104.84, 86.40, 84.78, 73.81, 73.66, 72.97, 71.81, 63.19, 50.12, 49.83, 40.27, 36.11, 26.65, 26.20, 25.92, 22.05, 21.97, 21.70, 21.65,18.33, 12.53, -5.39, -5.47; MS (FAB, NBA) m/z 616 $(M+1)^+$.

Phosphorothioate 2-17



To a mixture of **2-15** (20 mg, 0.03 mmol), T_5·OH (21 mg, 0.06 mmol) and 2-bromo-4,5-dicyanoimidazole (8.8 mg, 0.045 mmol) in a NMR tube was added dry acetonitrile (0.60 ml) at room temperature. The NMR tube was then sealed. ^{31}P NMR

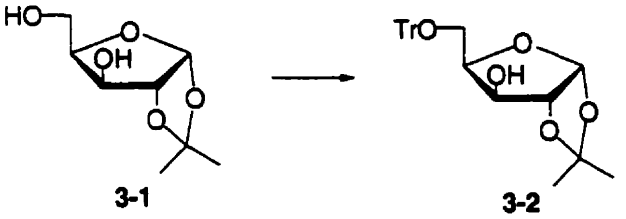
indicated the formation of two new resonances at 144.8 ppm and 143.7 ppm in a ratio of 6 to 1 within 10 minutes. Beaucage's reagent (7.2 mg, 0.036 mmol) was directly introduced to the reaction mixture. Instantaneously, the ^{31}P NMR showed peaks at 68.5 ppm and 68.7 in a ratio of 6 to 1. The mixture was purified by flash chromatography using ethyl acetate:hexanes:triethylamine (10:7:1) as eluant to afford the product as a white solid (24 mg, 72%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (m, 1H, ^{3}H-6), 7.23 (m, 1H, ^{5}H-6), 6.34-6.37 (m, 1H, ^{5}H-1'), 6.18 (m, 1H, ^{3}H-1'), 5.90 (d, 1H, H-1'', J = 3.42 Hz), 5.11-5.14 (m, 1H, ^{5}H-3'), 4.82-4.85 (dd, 1H, H-3'', J = 2.44 Hz and 12.2 Hz), 4.65 (d, 1H, H-2'', J = 3.91 Hz), 4.33-4.38 (m, 2H, ^{3}H-3', H-4''), 4.22-4.24 (m, 3H, 2x ^{3}H-5', ^{5}H-4'), 3.99 (m, 1H, ^{3}H-4'), 3.85 (s, 2H, ^{5}H-5'), 2.80-2.88 (m, 3H, H-5'', CHN), 2.43-2.46 (m, 1H, ^{5}H-2'), 2.10-2.27 (m, 3H, 2x ^{3}H-2', ^{5}H-2'), 1.91 (s, 3H, CH_3C=C), 1.89 (s, 3H, CH_3C=C), 1.27 (s, 3H, $C(CH_3)_2$, 1.25 (s, 3H, $C(CH_3)_2$,1.14 (m, 6H, $NHCH(CH_3)_2$, 0.91 (s, 9H, $SiC(CH_3)_3$), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 6H, $Si(CH_3)_2$), 0.06 (s, 6H, $Si(CH_3)_2$.

When catalysts **2-5**, or **2-8**, or **2-10** was used, the reactions were carried out in a similar fashion. When **2-5** or **2-8** was used, the coupling reactions took longer time (~ 2 hours) to go to completion.

7.3. Experimentals for Chapter 3

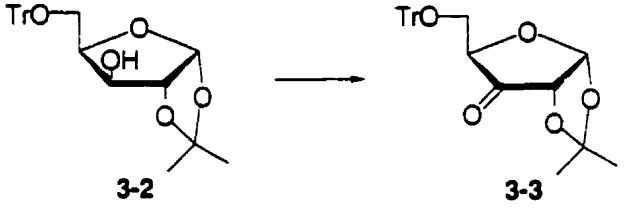
1,2-Di-O-isopropylidene-5-O-trityl-D-xylofuranose 3-2



Trityl chloride (5.58 g, 20 mmol) in dry dichloromethane was added slowly to a solution of 1,2-di-O-isopropylidene-D-xylofuranose **3-1** (3.80 g, 20 mmol) and triethylamine (3.07 ml, 22 mmol) in dry dichloromethane at 0 °C under argon. The resulting mixture was allowed to warm up to room temperature and stirred overnight. The crude mixture was washed with saturated ammonium chloride, water and brine, and dried over magnesium sulfate. Purification by flash chromatography afforded the desired product **3-2** (7.66 g, 90%) as a white solid.

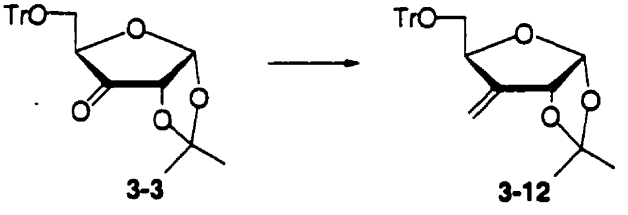
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.46 (m, 15H, phenyl protons), 6.01 (d, 1H, H-1, J_{1-2} = 3.5 Hz), 4.57 (d, 1H, H-2, J_{2-1} = 4.0 Hz), 4.24 (m, 2H, H-3, H-4), 3.46-3.59 (m, 2H, H-5), 3.17 (d, 1H, OH), 1.50 (s, 3H, $C(CH_3)_2$), 1.33 (s, 3H, , $C(CH_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 143.18, 128.44, 128.07, 127.29, 111.50, 104.96, 87.49, 85.08, 78.38, 76.29, 61.74, 26.76, 26.12.

1,2-Di-O-isopropylidene-5-O-trityl-D-xylofuranose –3-ulose 3-3



Chromium trioxide (1.2 g, 12 mmol) was added slowly to a stirred solution of pyridine (1.94 ml, 24 mmol) in sufficient amount of dichloromethane to dissolve $CrO_3 \bullet 2C_5H_5N$ complex. The mixture was stirred for 15 minutes to produce a deep red solution. Alcohol **3-2** (1.30 g, 3 mmol) dissolved in a small amount of dichloromethane was added with stirring. Acetic anhydride (1.13 ml, 12 mmol) was added at once. After 30 minutes, the mixture was transferred to the top of silica gel column, eluted with ethyl acetate. The solvent was then removed, coevaporated with toluene several times to remove any acetic anhydride and pyridine. Further drying under high vacuum furnished the desired ketone **3-3** as a white solid (1.26 g, 98%). m.p. 131 °C (lit.[236], 132 °C).

3-Deoxy-3-methylene-1,2-di-O-isopropylidene-5-O-trityl-D-Xylofuranose 3-12



3-3 **3-12**

To a suspension of methyl triphenylphosphoniumbromide (0.36 g, 1 mmol) in THF at –78 °C was added a 1.6 M *n*-butyllithium solution in hexane (0.69 ml, 1.1 mmol). The mixture was kept at –78 °C for half an hour and then allowed to warm up to 0 °C. Ketone **3-3** (0.43 g, 1 mmol) in THF was added. The mixture was allowed to warm up slowly to room temperature and stirred for one additional hour. The solvent was removed and the residue was taken up in ethyl acetate, washed with water, brine and dried over magnesium sulfate. Purification by flash chromatography afforded **3-12** as a white solid (0.36 g, 85%).

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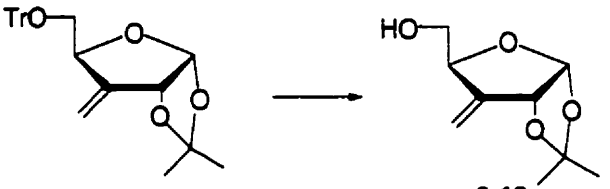
[236] Sowa. W. *Can. J. Chem.* **1986**, 46, 1586

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23-7.48 (m, 15H, phenyl protons), 5.99 (d, 1H, H-1, J_{1-2} = 4.0 Hz), 5.39 (m, 1H, C**H**$_2$=C), 5.08 (m, 1H, C**H**$_2$=C), 4.97 (d, 1H, H-2, J_{2-1} = 4.0 Hz), 4.92 (m, 1H, H-4), 3.20-3.31 (m, 2H, H-5), 1.55 (s, 3H, C(C**H**$_3$)$_2$), 1.43 (s, 3H, , C(C**H**$_3$)$_2$); MS (EI) m/z 428 (M^+).

3-Deoxy-3-methylene-1,2-di-O-isopropylidene-D-Xylofuranose 3-13

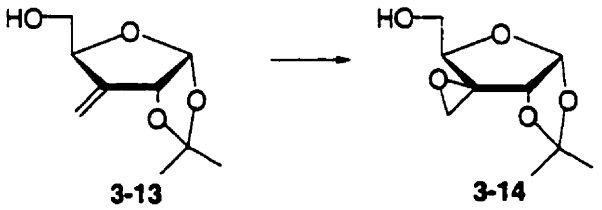


3-12 **3-13**

3-Deoxy-3-methylene-1,2-di-O-isopropylidene-5-O-trityl-D-Xylofuranose **3-12** (0.43 g, 1 mmol) was dissolved in 10% acetic acid in methanol (40 ml). The reaction mixture was brought to reflux for 12 hours. The pH of the solution was adjusted to around 7, extracted with chloroform several times. The organic extracts were washed with brine and dried over magnesium sulfate. Chromatographic purification furnished **3-13** as a colorless oil (0.12 g, 65 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84 (d, 1H, H-1, J_{1-2} = 4.0 Hz), 5.45 (m, 1H, CH_2=C), 5.16 (m, 1H, CH_2=C), 4.88 (d, 1H, H-2, J_{2-1} = 4.0 Hz), 4.79 (m, 1H, H-4), 3.85 (m, 1H, H-5), 3.64 (m, 1H, H-5), 1.97 (t, 1H, OH), 1.49 (s, 3H, C(C**H**$_3$)$_2$), 1.35 (s, 3H, C(C**H**$_3$)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 145.40 (**C**$(CH_3)_2$), 112.58 (CH_2=**C**), 112.20 (**C**H_2=C), 104.34 (C-1), 81.96 (C-2), 79.88 (C-4), 63.37 (C-5), 27.36 (C(**C**H_3)$_2$), 27.05 (C(**C**H_3)$_2$); MS (CI) m/z 187 ($(M+1)^+$, 22.6%), 155 (100%).

3-Epoxy-1,2-di-O-isopropylidene-D-Xylofuranose 3-14

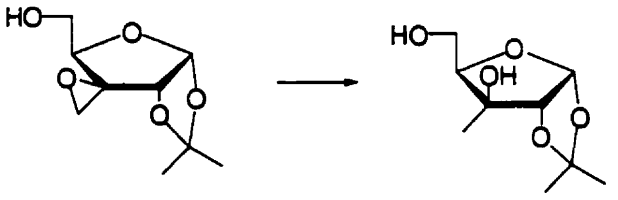


3-13 **3-14**

To 3-Deoxy-3-methylene-1,2-di-O-isopropylidene-D-Xylofuranose **3-13** (0.20 g, 1 mmol) in dichloromethane at room temperature was added 3-chloroperoxybenzoic acid (0.45 g, 1.5 mmol). The resulting mixture was then stirred at room temperature for 30 hours. The crude mixture was washed with 5% sodium thiosulfate solution, saturated sodium bicarbonate solution and brine, respectively. The aqueous layer was re-extracted with chloroform several times. The combined organic extracts were dried over magnesium sulfate. Purification by flash chromatography afforded the desired product **3-14** as a colorless oil (0.17 g, 82%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.02 (d, 1H, H-1, J_{1-2} = 3.5 Hz), 4.50 (m, 1H, H-4), 4.33 (d, 1H, H-2, J_{1-2} = 3.5 Hz), 3.62 (m, 2H, H-5), 3.16 (d, 1H, C**H**$_2$C(O)C, J = 3.5 Hz), 2.97 (d, 1H, C**H**$_2$C(O)C, J = 3.5 Hz), 1.52 (s, 3H, C(C**H**$_3$)$_2$), 1.32 (s, 3H, C(C**H**$_3$)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 133.51 (**C**(CH$_3$)$_2$), 104.04 (C-1), 84.03 (C-2), 76.40 (C-4), 65.34 (C-3), 60.22 (C-5), 46.42 (**C**(O)C), 26.93 (C(**C**H$_3$)$_2$), 26.58 (C(**C**H$_3$)$_2$); MS (EI) m/z 202 (M^+).

3-C-methyl-1,2-di-O-isopropylidene-D-xylofuranose 3-11

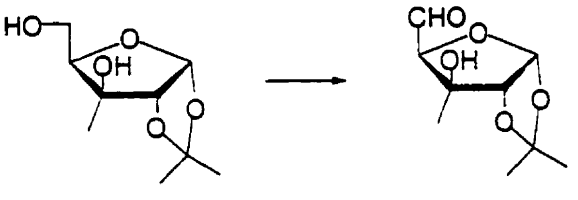


3-14 **3-11**

To a suspension of lithium aluminum hydride (31 mg, 0.82 mmol) in dry THF at 0 °C was added slowly epoxide **3-14** (0.17 g, 0.82 mmol) in THF. The reaction mixture was allowed to warm up to room temperature and stirred for 10 minutes. A few drops of ethyl acetate were added to the reaction mixture to destroy excess lithium aluminum hydride. A few drops of water (n) were added slowly, followed by 15% NaOH (n) and water (3n). The precipitate was washed with chloroform several times, the combined organic washings were dried over magnesium sulfate. The solvent was removed to afford **3-1** as white crystalline (0.13 g, 77%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 (d, 1H, H-1, J_{1-2} = 3.5 Hz), 4.24 (d, 1H, H-2, J_{1-2} = 3.5 Hz), 3.95-4.12 (m, 2H, H-5), 4.04 (br, 1H, OH), 2.24 (br, 1H, OH), 1.48 (s, 3H, $CH_3C(OH)$), 1.35 (s, 3H, $C(CH_3)_2$), 1.32 (s, 3H, , $C(CH_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 112.26, 104.54, 87.00, 80.74, 80.71, 60.21, 27.01, 26.29, 18.90; MS (CI) m/z 222 ($(M+1+NH_3)^+$, 33.8 %), 205 ($(M+1)^+$, 23.3%), 100 (100%).

3-C-methyl-1,2-di-O-isopropylidene-5-oxo-D-xylofuranose 3-15

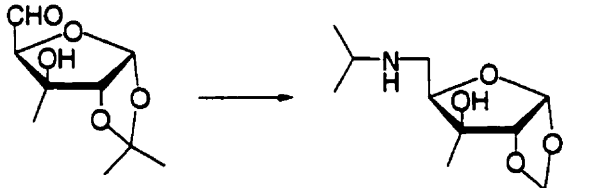


3-11 **3-15**

Dess-Martin reagent (0.138 g, 0.32 mmol) was added to the diol **3-11** (55 mg, 0.27 mmol) in dichloromethane at room temperature. The solution was stirred overnight. In the end of the reaction, silica gel was added directly into the reaction mixture. Evaporation of the solvent yielded the white solid mixture which was loaded directly on the top of the column and eluted with the mixture of ethyl acetate and hexane (1:1). The desired product was obtained as a colorless oil (46.4 mg, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.68 (s, 1H, CHO), 6.08 (d, 1H, H-1, $J_{1\text{-}2}$ = 3.5 Hz), 4.27 (d, 1H, H-2, $J_{1\text{-}2}$ = 3.5 Hz), 4.19 (s, 1H, H-4), 2.13 (br, 1H, OH), 1.51 (s, 3H, C(OH)C**H**$_3$), 1.49 (s, 3H, C(**CH**$_3$)$_2$), 1.34 (s, 3H, , C(**CH**$_3$)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 201.45, 113.06, 105.62, 86.92, 86.52, 82.44, 27.17, 26.44, 18.92; MS (CI) m/z 203 ((M+1)$^+$, 12.3 %), 173 (100%).

1,2-Di-O-isopropylidene-3-C-methyl-5-deoxyl-5-isopropylamino-D-xylofuranose 3-16



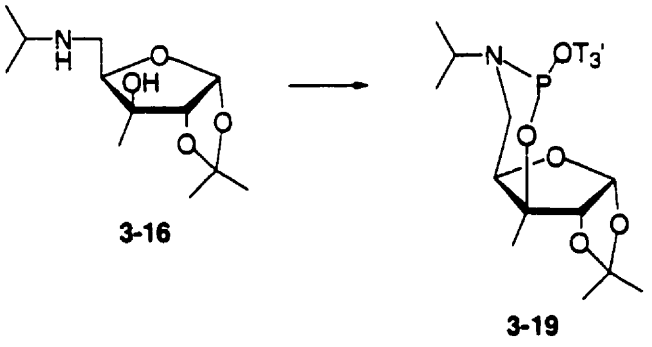
3-14 **3-16**

To a solution of aldehyde **3-14** (45 mg, 0.22 mmol) and isopropylamine (40 μl, 0.44 mmol) in dichloroethane (10 ml) was added sodium triacetoxyborohydride (70 mg, 0.33 mmol). The mixture was stirred at room temperature overnight. Aqueous sodium hydroxide (1N) was added to quench the reaction. The product was extracted by ether,

washed with brine and dried over magnesium sulfate. Purification by flash chromatography furnished **3-16** as a light amber oil (50 mg, 95%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (d, 1H, H-1, J_{1-2} = 3.5 Hz), 4.18 (d, 1H, H-2, J_{1-2} = 3.5 Hz), 3.84 (m, 1H, H-4), 3.25-3.28 (m, 1H, H-5), 2.83 (m, 1H, H-5); 2.71 (m, 1H, C**H**$(CH_3)_2$), 1.46 (s, 3H, C(OH)C**H**$_3$), 1.30 (s, 3H, C(C**H**$_3)_2$), 1.28 (s, 3H, , C(C**H**$_3)_2$), 1.04 (s, 3H, CH(C**H**$_3)_2$), 1.03 (s, 3H, CH(C**H**$_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.86, 104.84, 87.30, 80.64, 79.70, 48.56, 44.23, 27.09, 26.31, 22.66, 22.29, 19.59; MS (CI) m/z 246 ($(M+1)^+$, 93.9%).

Cyclic phosphoramidite 3-19



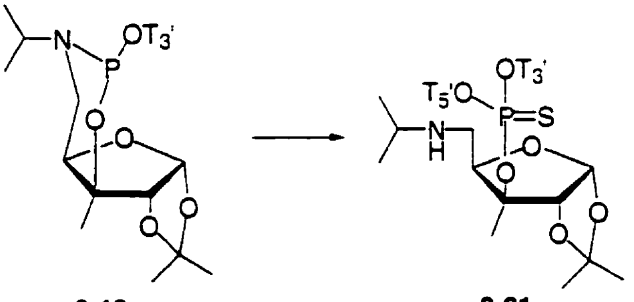
3-16 **3-19**

To a cooled solution of phosphorus trichloride (4.63 µl, 0.053 mmol) in dry dichloromethane (0.20 ml) in a NMR tube at 0 °C was added slowly the solution of **3-16** (13 mg, 0.053 mmol) in dry dichloromethane (0.45 ml) containing triethylamine (15.5 µl, 0.11 mmol). The NMR tube was then sealed. ^{31}P NMR showed a single resonance at around 149 ppm right after the addition. T_5·OH (20 mg, 0.058 mmol) in dry dichloromethane (0.4 ml) was then added. The NMR tube was re-sealed. A single resonance appeared at 132 ppm within five minutes. The reaction mixture was poured into a flask, the solvent was removed and the residue was directly separated by flash

chromatography by using a mixture of ethyl acetate and hexanes (1:7 + 20% triethylamine) as eluant to afford the desired product as a white solid (32 mg, 96%).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 133.36; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (s, 1H, H-6), 6.30 (m, 1H, H-1' of thymidine), 5.84 (d, 1H, H-1', $J_{1'-2'}$ = 3.5 Hz), 4.56 (m, 1H, H-3' of thymidine), 4.18 (d, 1H, H-2', $J_{1'-2'}$ = 3.0 Hz), 4.12 (m, 1H, H-4'), 3.93 (m, 1H, H-4' of thymidine), 3.77-3.90 (AB of ABX, 2H, H-5'), 3.43 (m, 1H, NC**H**$(CH_3)_2$), 3.33 (d, 1H, H-5' of thymidine, $J_{5'eq-5'ax}$ = 13.5 Hz), 3.03 (m, 1H, H-5' of thymidine), 2.35 (m, 1H, H-2' of thymidine), 2.05 (m, 1H, H-2' of thymidine), 1.89 (s, 3H, CH=C(C**H**$_3$)), 1.47 (d, 6H, , C(**CH**$_3)_2$), 1.09 (2d, 6H, NCH(C**H**$_3)_2$, J = 6.8 Hz and 7.8 Hz), 0.90 (s, 9H, SiC(C**H**$_3)_3$), 0.10 (s, 6H, Si(C**H**$_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 177.59, 163.70, 150.26, 135.26, 122.23, 110.86, 104.64, 86.81 (C-2', $^{3}J_{C2'-P}$ = 5.5 Hz), 86.30 (d, C-4', $^{3}J_{C4'-P}$ = 3.6 Hz), 84.90, 80.85 (d, C-3', $^{2}J_{C3'-P}$ = 8.2 Hz), 76.36, 73.59 (d, C-3' of thymidine, $^{2}J_{C3'-P}$ = 21 Hz), 63.19, 49.80 (d, N**C**H$(CH_3)_2$, $^{2}J_{C-P}$ = 37.7 Hz), 40.32 (d, C-2' of thymidine, $^{3}J_{C2'-P}$ = 5.5 Hz), 34.52 (d, C-5' of thymidine, $^{4}J_{C5'-P}$ = 14.5 Hz), 27.14, 25.93, 21.95 (d, NCH(**C**H$_3)_2$, $^{3}J_{C-P}$ = 10.1 Hz), 21.60 (d, NCH(**C**H$_3)_2$, $^{3}J_{C-P}$ = 6.4 Hz), 19.48, 12.51, 10.09, -5.38, -5.43; MS (FAB) m/z 630 ($(M+1)^+$, 40.4%), 504 (56.5%).

Dithymidine phosphorothioate

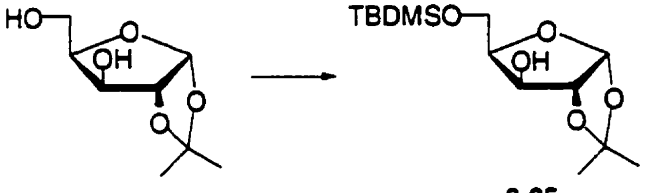


3-19 **3-21**

Phosphoramidite **3-19** (18 mg, 0.028 mmol), T_5-OH (15 mg, 0.03 mmol) and 2-bromo-4,5-dicyanoimidazole (13.7 mg, 0.07 mmol) were dried under vacuum overnight. Dry dichloromethane (0.45 ml) was added slowly to the mixture at 0 °C and the mixture was kept at 0 °C for 4 hours. Beaucage's sulfurizing (6.7 mg, 0.034 mmol) reagent was then added to the mixture. After 10 minutes, tetrabutylammonium fluoride (TBAF) was introduced and the reaction mixture was stirred at room temperature for 2 hours. The mixture was diluted with dichloromethane, washed with water and brine, and dried over magnesium sulfate. Purification by flash chromatography afforded the desired phosphorothioate as a white solid (8.8 mg, 40%).

^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (s, 1H), 7.53 (3, 1H), 6.26-6.30 (m, 2H), 5.98 (d, 1H, J = 3.5 Hz), 5.21 (m, 1H), 5.16 (d, 1H, J = 3.5 Hz), 4.29-4.38 (m, 3H), 4.20 (m, 1H), 4.07 (m, 2H), 3.79 (m, 3H), 3.03-3.12 (m, 2H), 2.95 (m, 1H), 2.48-2.51 (m, 1H), 2.15-2.40 (m, 3H), 1.92 (s, 3H), 1.88 (s, 3H), 1.72 (s, 3H), 1.48 (s, 3H), 1.31 (s, 3H), 1.17 (m, 6H); ^{31}P NMR (202.3 MHz, CD_3OD) δ 61.5, 61.3 (1:7); MS (FAB) 790 ($(M+1)^-$).

Synthesis of 1,2-di-O-isopropylidene-5-O-TBDMS-D-xylofuranose 3-25



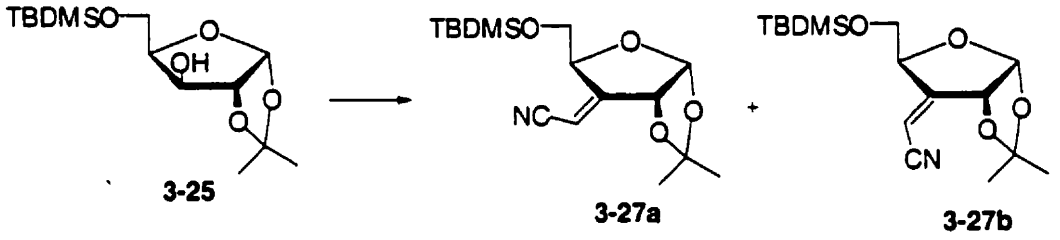
3-1 **3-25**

To 1,2-di-O-isopropylidene-D-xylofuranose **3-1** (10 g, 52.6 mmol) in dry DMF (100 ml) at 0 °C were added *tert*-butyldimethylsilyl chloride (8.71 g, 57.8 mmol) and imidazole (5.36 g, 78.9 mmol). The resulting mixture was stirred at 0 °C for 45 minutes and white precipitate was formed. The precipitate was filtered, and the filtrate was

evaporated to dryness under high vacuum. The residue was taken up in ethyl acetate, washed with water and brine, and dried over magnesium sulfate. The solvent was removed to afford the desired product as a colorless oil (15.8 g, 98.6%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (d, 1H, H-1, J_{1-2} = 3.5 Hz), 4.49 (d, 1H, H-2, J_{2-1} = 3.5 Hz), 4.32 (m, 1H, H-3), 4.10-4.13 (m, 3H, H-4 and H-5), 1.47 (s, 3H, $C(CH_3)_2$), 1.31 (s, 3H, $C(CH_3)_2$), 0.88 (s, 9H, $Si(CH_3)_3$), 0.09 (s, 6H, $Si(CH_3)_2(t\text{-Bu})$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.45, 104.95, 85.56, 78.08, 77.11, 62.38, 26.80, 26.13, 25.67, 18.10, -5.55, -5.66; MS (EI) m/z 304 (M^+).

3-Deoxy-3-cyanoethylene-1,2-O-isopropylidene-5-O-TBDMS-D-xylofuranose 3-27



Chromium oxide (6.83 g, 68.3 mmol) was added in portions into a stirred solution of pyridine (11 ml, 136.6 mmol) in sufficient amount of dichloromethane to dissolve CrO_3•2Py complex. The mixture was stirred for 15 minutes to produce a deep red solution. 1,2-di-O-isopropylidene-5-O-TBDMS-D-xylofuranose **3-25** (5.2 g, 17.1 mmol) in a small amount of dichloromethane was added with stirring at room temperature. Acetic anhydride (6.45 ml, 68.3 mmol) was added at once. After 30 minutes, the reaction mixture was concentrated to a small volume. The residue was loaded directly to the top of a column and eluted with ethyl acetate. The solvent was removed *in vacuo*, coevaporated

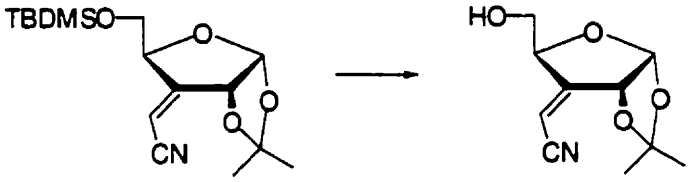
with toluene several times to remove pyridine and acetic anhydride. Further drying under high vacuum provided the crude ketone **3-26** (4.7 g) as a white solid.

To a suspension of sodium hydride (0.68 g, 17.1 mmol) in dry THF (15 ml) at 0 °C was added slowly diethylcyanomethyl phosphonate (2.76 ml, 17.1 mmol). The resulting mixture was allowed to stand at 0 °C for 45 minutes. 1,3-Di-O-isopropylidene-5-TBDMS-D-xylofuranose-3-ulose **3-26** (4.7 g, 15.6 mmol) in THF was added slowly. The reaction mixture was stirred at room temperature overnight. The mixture was then concentrated. The residue was extracted with ethyl acetate and washed with saturated sodium bicarbonate and water, and dried over magnesium sulfate. Purification by flash chromatography afforded the desired products **3-27a** (0.69 g, 12.4%) and **3-27b** (3.5 g, 62.0%), both as white solids.

E-isomer **3-27a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.98 (d, 1H, H-1, $J_{1\text{-}2}$ = 5 Hz), 5.69 (m, 1H, CNCH), 5.14 (m, 1H, H-4), 5.00 (m, 1H, H-2), 3.83-3.92 (m, 2H, H-5), 1.43 (s, 3H, $C(CH_3)_2$), 1.41 (s, 3H, $C(CH_3)_2$), 0.87 (s, 9H, $Si(CH_3)_3$), 0.06 (s, 3H, $Si(CH_3)_2$(*t*-Bu)), 0.05 (s, 3H, $Si(CH_3)_2$(*t*-Bu)).

Z-isomer **3-27b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (d, 1H, H-1, $J_{1\text{-}2}$ = 3.5 Hz), 5.65 (m, 1H, CNCH), 5.18 (m, 1H, H-2), 4.83 (m, 1H, H-4), 3.71-3.73 (m, 2H, H-5), 1.48 (s, 3H, $C(CH_3)_2$), 1.42 (s, 3H, $C(CH_3)_2$), 0.87 (s, 9H, $Si(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$(*t*-Bu)); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.42, 115.03, 113.82, 105.05, 96.12, 80.48, 80.13, 64.96, 27.41, 27.25, 25.74, 18.10, -5.50, -5.59; MS (CI) m/z 326 $(M+1)^+$.

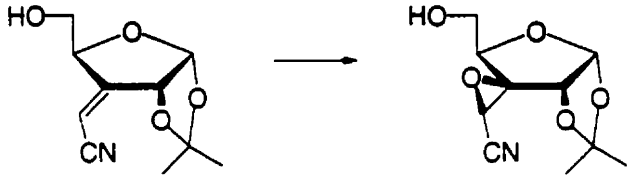
3-Deoxy-3-cyanoethylene-1,2-di-O-isopropylidene-D-xylofuranose 3-28



3-27b **3-28**

1M Tetrabutylammonium fluoride (TBAF) (6.71 ml, 6.71 mmol) was added slowly to **3-27b** (2.0 g, 6.1 mmol) in THF (10 ml) at 0 °C. The colorless solution turned dark during the addition. The dark solution was stirred at 0 °C for 10 minutes. The solvent was removed and the residue was taken up in ethyl acetate, washed with water and brine, and dried over magnesium sulfate. The crude was purified by flash chromatography to afford the desired product **3-28** (0.63 g, 49.2%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 (m, 1H, H-1), 5.53 (m, 1H, CNCH), 5.21 (m, 1H, H-2), 4.88 (m, 1H, H-4), 3.87 (m, 1H, H-5), 3.71 (m, 1H, H-5), 1.48 (s, 3H, C($\mathbf{CH_3}$)$_2$), 1.40 (s, 3H, C($\mathbf{CH_3}$)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 161.92, 114.60, 113.71, 104.42, 96.67, 79.76, 79.72, 62.67, 27.12, 26.99; MS (CI) m/z 212 ((M+1)$^+$, 14.2%), 180 (100%).

Epoxide 3-29



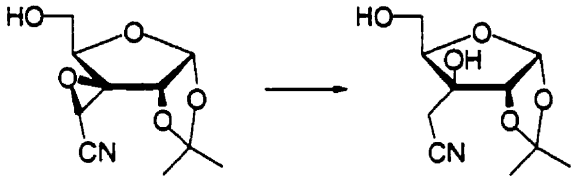
3-28 **3-29**

N-Benzyltrimethylammonium hydroxide (triton-B) (45.6 µl, 0.1 mmol) was added to *tert*-butyl hydroperoxide (TBHP) (0.29 ml, 1.5 mmol) in THF (5 ml) at 0 °C. After 1 minute, alkene **3-28** (0.21 g, 1 mmol) in a small amount of THF was added. The resulting

mixture was stirred for half an hour. Saturated ammonium chloride was added to the reaction mixture. The solvent was then removed, and the residue was taken up in ethyl acetate, washed with brine and dried over magnesium sulfate. Purification by flash chromatography yielded the desired product as a colorless oil (0.16 g, 72%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.02 (d, 1H, H-1, J = 3.5 Hz), 4.52-4.54 (m, 2H, H-2 and H-4), 3.89 (s, 1H, CNCH), 3.54-3.69 (m, 2H, H-5), 1.59 (s, 3H, $C(CH_3)_2$), 1.38 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 114.56, 113.84, 104.02, 82.38, 74.55, 70.32, 59.35, 41.42, 26.81, 26.45; MS (CI) m/z 230 (M+2H+1).

***Cis*-Diol 3-30**



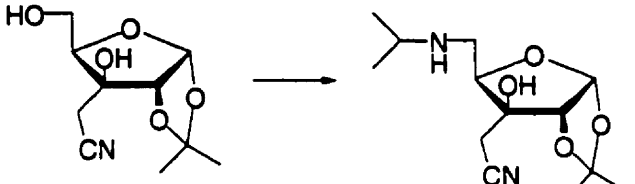
3-29 **3-30**

Lithium borohydride (13.5 mg, 0.62 mmol) was added to epoxide **3-29** (140 mg, 0.62 mmol) in 2-methoxylethyl ether (diglyme) (5 ml). The mixture was heated to 100 °C and stirred for 15 hours. The reaction mixture was then allowed to cool down to room temperature. Saturated ammonium chloride was added to quench the reaction. The solvent was removed under high vacuum, and the residue was taken up in ethyl acetate. Purification by flash chromatography afforded the desired product as a white solid (82 mg, 58 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 (d, 1H, H-1, J = 3.5 Hz), 5.32 (br, 1H, OH), 4.39 (d, 1H, H-2, J = 3.5 Hz), 4.19 (m, 2H, H-5), 3.89 (m, 1H, H-4), 2.73-2.85 (2d's, 2H, CH_2CN, J = 16.5 Hz, AB system), 1.49 (s, 3H, $C(CH_3)_2$), 1.33 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (125.7

MHz, $CDCl_3$) δ 116.67, 112.95, 104.06, 85.91, 80.05, 78.87, 60.48, 26.82, 26.26, 23.13; MS (FAB, NBA) m/z 230 $(M+1)^+$.

1,2-Di-O-isopropylidene-3-C-cyanoethyl-5-deoxy-5-isopropylamino-xylofuranose 3-32



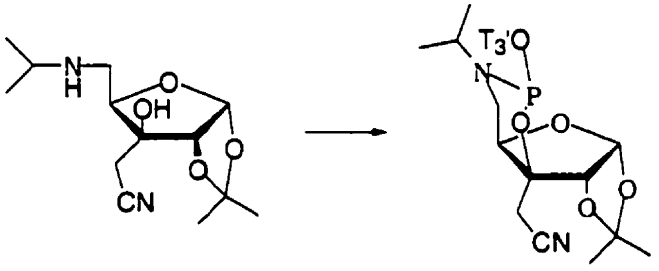
3-30 **3-32**

A solution of oxalyl chloride (12.8 µl, 0.14 mmol) in dichloromethane (5 ml) was cooled down to – 78 °C and treated dropwise with DMSO (19 µl, 0.26 mmol) under argon. After 15 minutes, a solution of diol **3-30** (28 mg, 0.12 mmol) in dry dichloromethane (5 ml) was slowly introduced. The mixture was stirred for 30 minutes at – 78 °C. Triethylamine (85 µl, 0.6 mmol) was introduced and the reaction mixture was stirred for an additional 30 minutes. The mixture was then allowed to warm up slowly to room temperature. The solvent was removed *in vacuo* to give the crude aldehyde **3-31**. The aldehyde **3-31** was dissolved in dichloroethane (DCE) (10 ml). Excess isopropylamine and sodium triacetoxyborohydride were then added. The mixture was stirred at room temperature for 20 hours. Aqueous sodium hydroxide (2N) was introduced to quench the reaction. Aqueous layer was extracted with ether, the combined extracts were washed with brine and dried over magnesium sulfate. Purification by flash chromatography (using 1:1 ethyl acetate:hexanes to 5:5:1 ethyl

acetate:hexanes:triethylamine as eluents) afforded the desired aminoalcohol **3-32** as a white solid (25 mg, 77% for two steps).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (d, 1H, H-1, J = 3.5 Hz), 4.29 (d, 1H, H-2, J = 3.5 Hz), 3.92 (m, 1H, H-4), 3.35-3.39 (m, 2H, H-5), 2.78 (sept, 1H, NC**H**$(CH_3)_2$), 2.63-2.78 (2d's, 2H, C**H**$_2$CN, J = 16.5 Hz, AB system), 1.46 (s, 3H, C(C**H**$_3)_2$), 1.30 (s, 3H, C(C**H**$_3)_2$),1.05-1.09 (2d's, 6H, , NCH(C**H**$_3)_2$, J = 6.5 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 117.26, 112.58, 104.21, 86.56, 80.24, 77.93, 48.67, 44.99, 26.93, 26.29, 23.74, 22.58, 22.33; MS (CI) m/z 271 ($(M+1)^+$, 100%).

Cyclic Phosphoramidite 3-34



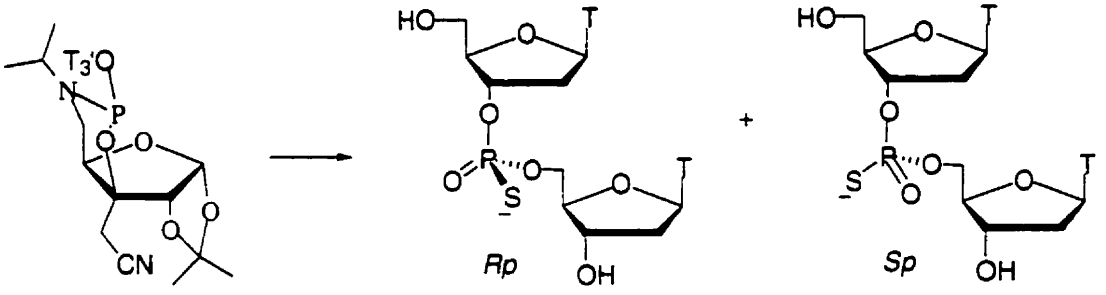
3-32 **3-34**

To a cooled solution of phosphorus trichloride (6.78 µl, 0.077 mmol) in dry dichloromethane (0.20 ml) in a NMR tube at 0 °C was added slowly a solution of **3-32** (21 mg, 0.077 mmol) in dry dichloromethane (0.45 ml) containing triethylamine (23.8 µl, 0.17 mmol). The NMR tube was then sealed. ^{31}P NMR showed a major resonance at 147.6 ppm right after the addition. A solution of 5'-O-TBDMS-thymidine (30.5 mg, 0.085 mmol) in dry dichloromethane (0.4 ml) containing triethylamine (11.8 µl. 0.085 mmol) was then added. The NMR tube was re-sealed. A single resonance appeared at 133 ppm within five minutes. The solvent was removed and the residue was purified by flash

column chromatography by using ethyl acetate/hexanes/triethylamine (10/20/3) as eluant to afford the desired phosphoramidite **3-34** as a white solid (40 mg, 81%).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 133.08; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (s, 1H, H-6), 6.29 (m, 1H, H-1' of thymidine), 5.84 (d, 1H, H-1'', J = 3.5 Hz), 4.66 (m, 1H, H-3' of thymidine), 4.41 (d, 1H, H-2'', J = 3.5 Hz), 4.20 (m, 1H, H-4'), 3.88-3.94 (m, 3H, H-4'' and H-5'), 3.48 (sept, 1H, NC**H**$(CH_3)_2$), 3.27-3.31(m, 1H, H-5''), 3.05-3.10 (m, 2H, H-5'', C**H**$_2$CN), 2.81 (d, 1H, CH_2CN, J = 16 Hz), 2.36 (m, 1H, H-1'), 2.09 (m, 1H, H-2'), 1.90 (s, 3H, CH=C(C**H**$_3$)), 1.48 (s, 3H, , C(C**H**$_3$)$_2$), 1.32 (s, 3H, , C(C**H**$_3$)$_2$), 1.12 (d, 6H, NCH(C**H**$_3$)$_2$, J = 7 Hz), 0.90 (s, 9H, SiC(C**H**$_3$)$_3$); 0.10 (s, 6H, Si(C**H**$_3$)$_2$(*t*-Bu); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.55, 150.18, 135.26, 116.34, 113.00, 110.89, 104.45, 86.65 (J = 4.5 Hz), 84.87, 84.82, 78.83,78.76, 75.08, 74.89, 74.28, 63.30, 50.32, 50.01, 47.49, 45.95, 40.60 (d, J = 4.5 Hz), 39.93, 34.39 (d, J = 3.6 Hz), 26.95, 26.42, 25.94, 23.21, 22.04 (d, J = 9.1 Hz), 21.74 (d, J = 5.4 Hz), 18.31, 12.51, -5.39, -5.43; MS (FAB, NBA) m/z 655 $(M+1)^+$.

Dithymidine phosphorothioates



3-34 **3-37** **3-38** (major)

Phosphoroamide **3-34** (30 mg, 0.046 mmol), T_5-OH (19.6 mg, 0.055 mmol) and 2-bromo-4,5-dicyanoimidazole (18 mg, 0.09 mmol) were dried under vacuum overnight.

Dry dichloromethane (0.45 ml) was added slowly to the mixture at 0 °C and the mixture was kept at that temperature under argon overnight. Beaucage's reagent (11 mg, 0.055 mmol) was added to the mixture. After 5 minutes, concentrated ammonium hydroxide (1 ml) was added. Tetrabutylammonium fluoride (TBAF) (1 ml) was introduced after 10 minutes and the reaction mixture was stirred at room temperature for 1 hour. The crude mixture was purified by flash chromatography to afford the desired phosphorothioates **3-37** (*Rp*) and **3-38** (*Sp*) (1:6) as white solids (12.2 mg, 33%).

3-38 (*Sp*): ^{31}P NMR (202.3 MHz, CD_3OD) δ 58.86; ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (s, 1H, ^{5}H-6), 7.78 (s, 1H, ^{3}H-6), 6.29 (dd, 1H, ^{5}H-1', J = 8.0 Hz and 6.5 Hz), 6.22 (dd, 1H, ^{3}H-1', J = 7.8 Hz and 6.0 Hz), 4.98 (m, 1H, ^{3}H-3'), 4.45 (m, 1H, ^{5}H-3'), 4.12 (m, 1H, ^{3}H-4'), 4.05 (m, 2H, ^{5}H-5'), 3.98 (m, 1H, ^{5}H-4'), 3.74 (m, 2H, ^{3}H-5'), 3.17 (m, 8H), 2.40 (m, 1H, ^{3}H-2'), 2.20 (m, 2H, ^{5}H-2'), 2.12 (m, 1H, ^{5}H-2'), 1.90 (s, 3H, CH=C(C**H**$_3$)), 1.80 (s, 3H, CH=C(C**H**$_3$)); 1.59 (m, 8H), 1.35 (m, 8H), 0.94 (m, 12H); MS (FAB-, NBA) m/z 561 $(dTP(S)(O)dT)^-$; HPLC: Retention time: 14.62 min; (Phenomenex C8 Column; solvent A: water; solvent B: acetonitrile; flow rate: 1.5 ml/min; 3% B increases linearly to 7% for the first 30 minutes, then increases to 40% during the next 20 minutes).

3-37 (*Rp*): ^{31}P NMR (202.3 MHz, CD_3OD) δ 58.81; HPLC: Retention time: 10.04 min, conditions same as the above.

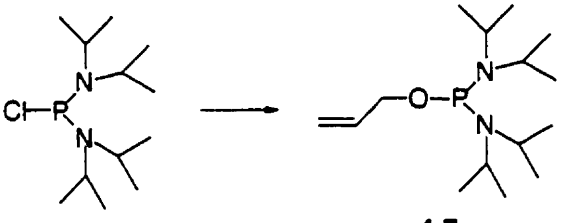
7.4. Experimentals for Chapter 4

Bis(diisopropylamino)chlorophosphine

$$PCl_3 + 4\ NH(i\text{-}Pr)_2 \rightarrow ClP(N(i\text{-}Pr)_2)_2$$

A solution of phosphorus trichloride (13 ml, 0.15 mol) in dry ether (300 ml) was added dropwise into a solution of diisopropylamine (84 ml, 0.6 mol) in dry ether (250 ml) at –78 °C. After half an hour, the reaction mixture was warmed up and brought to reflux. When ^{31}P NMR showed a major peak at around 134 ppm, the reaction mixture was cooled down to room temperature. The precipitate was filtered off and washed with ether. Ether was removed by an argon flow and the residue was used in next reaction without any further purification.

Allyl *N,N,N',N'*-tetraisopropylphosphorodiamidite 4-7

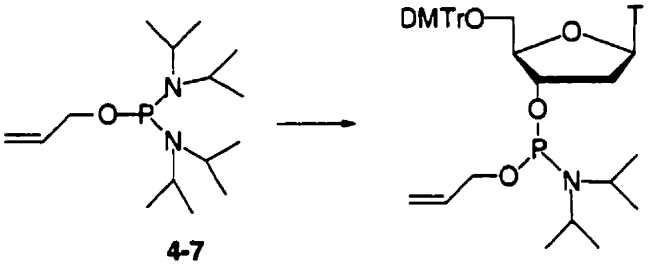


4-7

To a solution of bis(diisopropylamino)chlorophosphine obtained from the previous experiment in dry ether (200 ml) at 0 °C were added triethylamine (15.4 ml) and allyl alcohol (7.48 ml). The reaction mixture was then gradually warmed up and stirred at room temperature. ^{31}P NMR indicated the formation of a new peak at about 125 ppm. At the end of reaction, the original peak at 134 ppm shifted to 125 ppm completely. The

precipitate was filtered. The filtrate was concentrated and the residue went *in situ* to the next step.

5'-O-(4,4')-(Dimethoxytrityl)-3'-(allyloxy diisopropylamino phosphinyl) thymidine 4-8



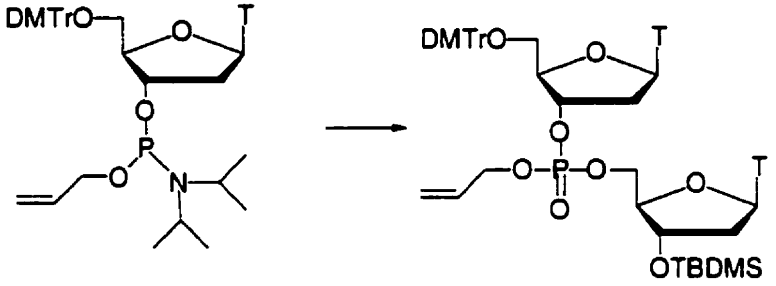
4-7 **4-8**

To a suspension of 5'-O-DMTr-thymidine (2.2 g, 4 mmol) in dry acetonitrile were added diisopropylamine (0.62 ml, 4.4 mmol), **4-7** (1.9 ml, 6 mmol) and tetrazole (0.31 g, 4.4 mmol). After stirring at room temperature for one and a half hours, the solvent was removed *in vacuo*. The residue was taken up in ethyl acetate. The combined organic extracts were washed with brine and dried over magnesium sulfate. Column chromatographic separation afforded **4-8** as a white solid (2.6 g, 89%).

m.p. 66-69 °C; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 148.6, 148.1; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.36 (br, 1H, NHCO), 7.62(m, 1H, C**H**=C(CH_3)), 7.19-7.40 (m, 9H, aromatic protons of DMTr), 6.81 (d, 4H, J = 8.64 Hz. protons *ortho* to OCH_3 of DMTr), 6.40 (m, 1H, H-1'), 5.84 (m, 1H, $CH_2C\mathbf{H}=CH_2$), 5.02-5.30 (m, 2H, $CH_2CH=C\mathbf{H_2}$), 4.65 (m, 1H, H-3'), 3.91 - 4.19 (m, 3H, $C\mathbf{H_2}CH=CH_2$ and H-4'), 3.77 (s, 6H, 2 OCH_3's of

DMTr), 3.28-3.64 (m, 4H, 2xC**H**$(CH_3)_2$, H-5'), 2.23-2.48 (m, 2H, H-2'), 1.39 (s, 3H, CH_3); MS (FAB) m/z 732 ($(M+1)^+$).

Allyl-5'-O-(4,4'-dimethoxytrityl)-(3'-5')-3'-TBDMS dithymidine phosphate 4-5O



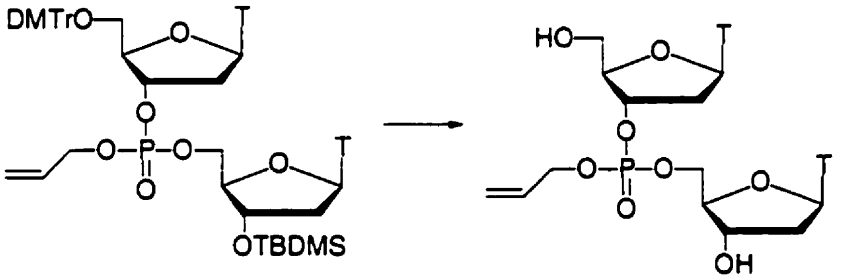
4-8 **4-5O**

Phosphoramidite **4-8** (1.46 g, 2 mmol), 3'-O-TBDMS-thymidine (0.78 g, 2.2 mmol) and tetrazole (0.15 g, 2.2 mmol) were dried under vacuum overnight. The flask containing reagents was flushed with argon and stopped by a rubber septum. Fresh distilled acetonitrile (20 ml) was added. The resulting solution was stirred room temperature overnight. Iodine (1.1 g, 4 mmol) in lutidine/THF/H_2O (2/2/1) was added. After 5 minutes, the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane, washed with 5% sodium thiosulfate. The aqueous layer was back extracted with dichloromethane several times. The combined organic layers were washed with brine, and dried over magnesium sulfate. Purification by flash chromatography afforded the desired **4-5O** (1.28 g, 63%) as a white solid.

^{31}P NMR (202.3 MHz, $CDCl_3$) δ -1.17, -1.32; ^1H NMR (500 MHz, $CDCl_3$) δ 8.34-8.56 (m, 2H), 7.23-7.36 (m, 9H), 6.83 (d, 4H, J = 8.5 Hz), 6.42-6.45 (m, 1H), 6.20-6.25 (m, 1H), 5.80-5.91 (m, 1H), 5.17-5.38 (m, 2H), 4.14-4.56 (m, 5H), 3.96-3.99 (m, 1H), 3.79

(s, 6H), 3.51 (m, 1H), 3.34-3.39 (m, 1H), 2.59-2.64 (m, 1H), 2.38-2.45 (m, 1H), 2.08-2.30 (m, 4H), 1.89 (s, 3H), 1.36 (s, 3H), 0.88 (2s's, 9H), 0.06-0.08 (m, 6H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.44, 163.39, 158.80, 150.24, 150.10, 143.96, 143.91, 135.72, 135.53, 135.09, 134.94, 134.88, 131.79, 131.74, 131.69, 130.09, 128.09, 128.06, 127.32, 119.41, 119.18, 113.31, 111.78, 111.71, 111.26, 111.15, 87.30, 85.53, 85.21, 82.88, 84.65, 84.26, 84.20, 79.14, 71.31, 68.77, 63.29, 55.25, 39.03, 25.65, 12.45, 11.60, -4.66, -4.69.

Allyl-3'-5'-dithymidine phosphate 4-6O



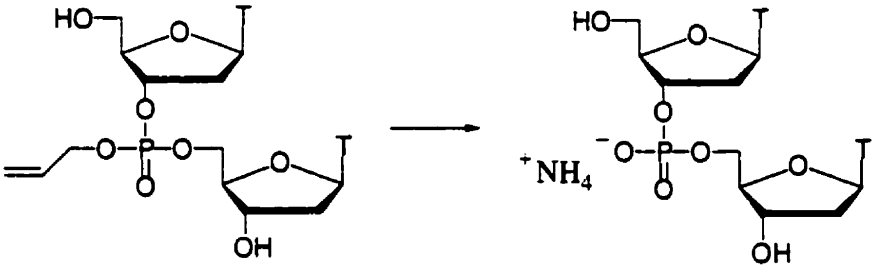
4-5O **4-6O**

A solution of phosphate **4-5O** (1.02 g, 1 mmol) in 80% aqueous acetic acid (10 ml) was stirred at room temperature until TLC showed the disappearance of the starting material. The pH of the solution was adjusted to around 7, extracted with ethyl acetate. The combined organic extracts were washed with water, and dried over magnesium sulfate. The solvent was removed *in vacuo*. To the residue was added 1M TBAF (1 ml) in THF. The solution was stirred at room temperature for 2 hours. Purification by flash chromatography furnished the desired product **4-6O** (0.43g, 74%) as a white solid.

(5'-hydroxyl-thymidine was referred as A, 3'-hydroxyl-thymidine was referred as B)

^{31}P NMR (202.3 MHz, D_2O) δ -1.22, -1.37; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (two q's, 1H, C**H**=C(CH_3), $^{4}J_{H-H}$ = 1.0 Hz), 7.53 (two q's, 1H, C**H**=C(CH_3), $^{4}J_{H-H}$ = 1.0 Hz), 6.27 (m, 2H, H-1'A, H-1'B), 6.00 (m, 1H, CH_2=C**H**-CH_2-O), 5.40 (m, 1H, C**H$_2$**=CH-CH_2-O), 5.29 (m, 1H, C**H$_2$**=CH-CH_2-O), 5.08 (m, 1H, H-3'A), 4.63 (m, 2H, CH_2=CH-C**H$_2$**-O), 4.41 (m, 1H, H-3'B), 4.31 (m, 2H, H-5'B), 4.20 (m, 1H, H-4'A), 4.04 (m, 1H, H-4'B), 3.77 (m, 2H, H-5'A), 2.51 (m, 1H, H-2'A), 2.36 (m, 1H, H-2'A), 2.27 (m, 2H, H-2'B), 1.89 (d, 3H, CH=C(C**H$_3$**), $^{4}J_{H-H}$ = 1.0 Hz), 1.87 (two d's, 3H, CH=C(C**H$_3$**), $^{4}J_{H-H}$ = 1.0 Hz); ^{13}C NMR (125.7 MHz, CD_3OD) δ 166.31 (2xNHCOC(CH_3)), 152.35, 152.32(2xNCONH), 137.83, 137.81(2xCH=C(CH_3)), 133.55(two d's, CH_2=CH-CH_2-O, $^{3}J_{C-P}$ = 3.65 Hz), 119.37, 119.29 (CH=CH-CH_2-O), 111.93, 111.89 (2xNCH=C(CH_3)), 87.12 (two d's, C-4'A, $^{3}J_{4'A-P}$ = 2.76 Hz, $^{3}J_{4'A-P}$ = 1.9 Hz), 86.62 (C-1'A), 86.54 (C-1'B), 86.03 (two d's, C-4'B, $^{3}J_{3'B-P}$ = 3.65 Hz, $^{3}J_{3'B-P}$ = 6.4 Hz), 80.61 (two d's, C-3'A, $^{2}J_{3'A-P}$ = 5.4 Hz), 71.72, 71.62 (C-3'B), 70.16 (two d's, CH_2=CH-CH_2-O, $^{2}J_{C-P}$ = 4.5 Hz, $^{2}J_{C-P}$ = 5.5 Hz), 68.88 (two d's, C-5'B, $^{2}J_{5'B-P}$ = 6.4 Hz), 62.60, 62.57 (C-5'A), 40.48, 40.44 (C-2'B), 39.51(two d's, C-2'A, $^{3}J_{2'A-P}$ = 3.65 Hz, $^{3}J_{2'A-P}$ = 2.76 Hz), 12.54, 12.46 (2xCH=C(CH_3)).

Ammonium phosphate 4-9O



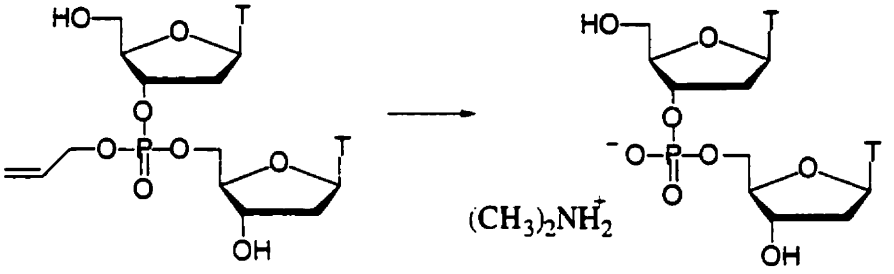
4-6O **4-9O**

A solution of the allyl protected **4-6O** (20 mg, 0.034 mmol) in concentrated aqueous ammonia (5 ml) was heated at 55 °C for 2 hours. Water was removed by high vacuum and the product **4-9O** (18 mg, 95%) was obtained as a white solid.

^{31}P NMR (202 MHz, D_2O) δ -1.68; ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 (q, 1H, C**H**=C(CH_3), $^{4}J_{H\text{-}H}$ = 1.0 Hz), 7.77 (q, 1H, C**H**=C(CH_3), $^{4}J_{H\text{-}H}$ = 1.0 Hz), 6.33 (dd, 1H, H-1'B, $^{3}J_{1'H\text{-}2'H}$ = 6.5 Hz, $^{3}J_{1'H\text{-}2'H}$ = 8.0 Hz), 6.28 (dd, 1H, H-1'A, $^{3}J_{1'H\text{-}2'H}$ = 5.5 Hz, $^{3}J_{1'H\text{-}2'H}$ = 7.5 Hz), 4.85 (m, 1H, H-3'A), 4.48 (m, 1H, H-3'B), 4.13 (m, 1H, H-4'A), 4.07 (m, 2H, H-5'B), 4.01 (m, 1H, H-4'B), 3.78 (d, 2H, H-5'A, $^{3}J_{5'H\text{-}4'H}$ = 3.0 Hz), 2.47 (m, 1H, H-2'A), 2.18-2.30 (m, 3H, H-2'A, H-2'B), 1.92 (d, 3H, CH=C(C**H**$_3$), $^{4}J_{H\text{-}H}$ = 1.0 Hz), 1.86 (d, 3H, CH=C(C**H**$_3$), $^{4}J_{H\text{-}H}$ = 1.0 Hz); ^{13}C NMR (125.7 MHz, CD_3OD) δ 167.02, 166.99 (2xNHCOC(CH_3)), 152.86, 152.77 (2xNCONH), 138.08, 137.99 (2xCH=C(CH_3)), 112.01, 111.61 (2xNCH=C(CH_3)), 87.76 (d, C-4'A, $^{3}J_{4'A\text{-}P}$ = 5.5 Hz), 87.31 (d, C-4'B, $^{3}J_{4'B\text{-}P}$ = 9.1 Hz), 86.24 (C-1'A), 86.00 (C-1'B), 76.77 (d, C-3'A, $^{2}J_{2'A\text{-}P}$ = 5.5 Hz), 72.53 (C-3'B), 66.33 (d, C-5'B, $^{2}J_{5'B\text{-}P}$ = 5.5 Hz), 62.77 (C-5'A), 40.80

(C-2'B), 40.07 (d, C-2'A, $^3J_{2'A\text{-}P}$ = 2.64 Hz), 12.69, 12.53 (2xCH=C(CH_3)); MS (FAB-, NBA) m/z 545((dTP(O)dT)$^{-1}$).

Dimethylammonium phosphate 4-9O

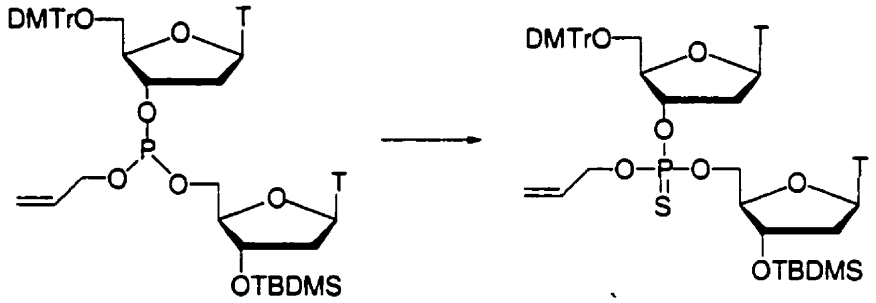


4-6O **4-9O**

A solution of the allyl protected **4-6O** (20 mg, 0.034 mmol) in 40% aqueous dimethylamine (5 ml) was stirred at room temperature for half an hour. Water was then removed by high vacuum to afford the product **4-9O** (19.3 mg, 96%) as a white solid. ^{31}P NMR (202 MHz, D_2O) δ -1.65; ^{1}H NMR (500 MHz, CD_3OD) δ 7.84 (q, 1H, C**H**=C(CH_3), $^4J_{H\text{-}H}$ = 1.0 Hz), 7.78 (q, 1H, C**H**=C(CH_3), $^4J_{H\text{-}H}$ = 1.0 Hz), 6.32 (dd, 1H, H-1'B, $^3J_{1'H\text{-}2'H}$ = 5.5 Hz, $^3J_{1'H\text{-}2'H}$ = 7.5 Hz), 6.30 (dd, 1H, H-1'A, $^3J_{1'H\text{-}2'H}$ = 6.0 Hz, $^3J_{1'H\text{-}2'H}$ = 8.0 Hz), 4.87 (m, 1H, H-3'A), 4.48 (m, 1H, H-3'B), 4.13 (m, 1H, H-4'A), 4.07 (m, 2H, H-5'B), 4.01 (m, 1H, H-4'B), 3.78 (d, 2H, H-5'A, $^3J_{5'H\text{-}4'H}$ = 3.0 Hz), 2.72 (s, 6H, N(CH_3)$_2$), 2.47 (m, 1H, H-2'A), 2.18-2.30 (m, 3H, H-2'A, H-2'B), 1.93 (d, 3H, CH=C(C**H$_3$**), $^4J_{H\text{-}H}$ = 1.0 Hz), 1.86 (d, 3H, CH=C(C**H$_3$**), $^4J_{H\text{-}H}$ = 1.0 Hz); ^{13}C NMR (125.7 MHz, CD_3OD) δ 166.46, 166.42 (2xNHCOC(CH_3)), 152.44, 152.29 (2xNCONH), 138.15, 138.05 (2xCH=C(CH_3)), 111.98, 111.56 (2xNCH=C(CH_3)),

87.80 (d, C-4'A, $^{3}J_{4'A\text{-}P}$ = 6.4 Hz), 87.33 (d, C-4'B, $^{3}J_{4'B\text{-}P}$ = 8.3 Hz), 86.20 (C-1'A), 86.05 (C-1'B), 76.84 (d, C-3'A, $^{2}J_{2'A\text{-}P}$ = 4.5 Hz), 72.52 (C-3'B), 66.35 (d, C-5'B, $^{2}J_{5'B\text{-}P}$ = 5.5 Hz), 62.78 (C-5'A), 40.80 (C-2'B), 40.08 (d, C-2'A, $^{3}J_{2'A\text{-}P}$ = 3.6 Hz), 35.38 ($NH(CH_3)_2$), 12.63, 12.46 (2xCH=C(CH_3)); MS (FAB-, NBA) m/z 545($(dTP(O)dT)^{-1}$).

Allyl-5'-O-(4,4'-dimethoxytrityl)-(3'-5')-3'-TBDMS dithymidine phosphorothioate 4-5S



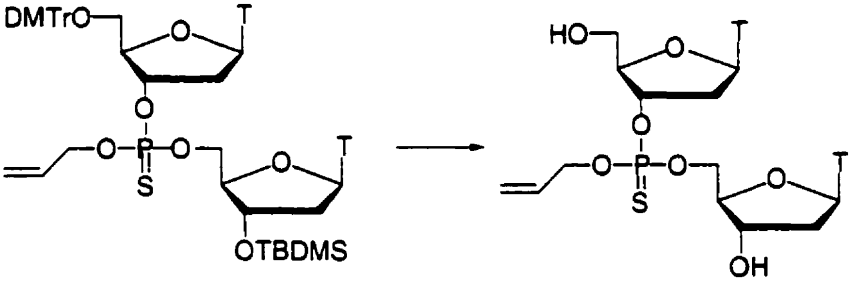
4-8 **4-5S**

Phosphoramidite **4-8** (1.46 g, 2 mmol), 3'-O-TBDMS-thymidine (0.78 g, 2.2 mmol) and tetrazole (0.15 g, 2.2 mmol) were dried under vacuum overnight. The flask containing reagents was flushed with argon and stopped by a rubber septum. Fresh distilled acetonitrile (20 ml) was added. The resulting solution was stirred room temperature overnight. Beaucage's reagent (0.44 g, 2.2 mmol) was added. After 5 minutes, the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane, washed with 5% sodium thiosulfate, the aqueous layer was back extracted with dichloromethane several times. The combined organic layers were washed

with brine, and dried over magnesium sulfate. Purification by flash chromatography afforded the desired **4-5S** (1.37 g, 66%) as a white solid.

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 68.86, 68.53; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23-7.38 (m, 9H), 6.83 (d, 4H, J = 8.5 Hz), 6.40-6.43 (m, 1H), 6.20-6.25 (m, 1H), 5.77-5.92 (m, 1H), 5.18-5.37 (m, 2H), 4.40-4.50 (m, 3H), 4.02-4.26 (m, 5H), 3.78 (s, 6H), 3.41 (m, 2H), 2.56-2.59 (m, 1H), 2.38 (m, 1H), 2.37 (m, 1H), 2.26 (m, 1H), 1.91 (2s's, 3H), 1.43 (2s's, 3H), 0.88 (2s's, 9H), 0.08 (m, 6H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.89, 163.75, 158.58, 150.38, 150.27, 143.94, 135.47, 134.99, 134.86, 131.59, 131.54, 129.87, 127.92, 127.86, 127.08, 118.96, 113.19, 111.59, 111.03, 87.08, 85.23, 84.88, 84.81, 84.48, 84.43, 84.18, 79.43, 79.39, 71.48, 69.05, 69.01, 66.88, 66.84, 63.17, 55.09, 46.53, 38.94, 25.53, 12.41, 11.59, -4.78, -4.96.

Allyl-3'-5'-dithymidine phosphorothioate 4-6S



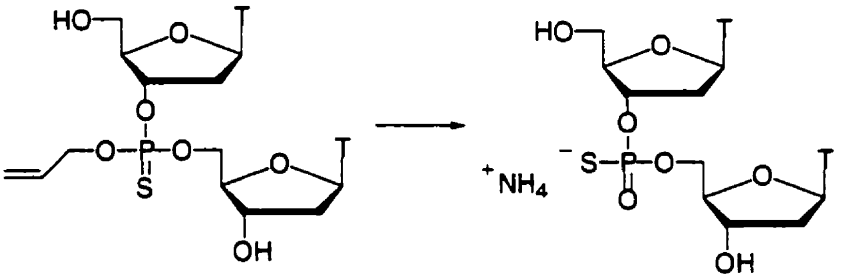
4-5S **4-6S**

A solution of phosphate **4-5S** (1.04 g, 1 mmol) in 80% aqueous acetic acid (10 ml) was stirred at room temperature until TLC showed the disappearance of the starting material. The pH of the solution was adjusted to around 7, extracted with ethyl acetate. The combined organic extracts were washed with water, and dried over magnesium

sulfate. The solvent was removed *in vacuo*. To the residue was added 1M TBAF (1 ml) in THF. The solution was stirred at room temperature for 2 hours. Purification by flash chromatography furnished the desired product **4-6S** (0.46 g, 76%) as a white solid.

Compound ^{31}P NMR (202.3 MHz, D_2O) δ 68.49, 68.37; ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (two q's, 1H, C**H**=C(CH_3), $^4J_{H-H}$ = 1.5 Hz), 7.58 (two q's, 1H, C**H**=C(CH_3), $^4J_{H-H}$ = 1.0 Hz), 6.28 (m, 2H, H-1'A, H-1'B), 5.98 (m, 1H, CH_2=C**H**-CH_2-O), 5.38 (m, 1H, C**H$_2$**=CH-CH_2-O), 5.26 (m, 1H, C**H$_2$**=CH-CH_2-O), 5.19 (m, 1H, H-3'A), 4.62 (m, 2H, CH_2=CH-C**H$_2$**-O), 4.41 (m, 1H, H-3'B), 4.31(m, 2H, H-5'B), 4.20 (m, 1H, H-4'A), 4.06 (m, 1H, H-4'B), 3.77 (m, 2H, H-5'A), 2.48 (m, 1H, H-2'A), 2.19-2.37 (m, 3H, H-2'A, H-2'B), 1.91 (s, 3H, CH=C(C**H$_3$**)), 1.87 (two d's, 3H, CH=C(C**H$_3$**)); ^{13}C NMR (125.7 MHz, CD_3OD) δ 166.38, 166.34 (2xNH**C**OC(CH_3)), 152.36, 152.30 (2xN**C**ONH), 137.88, 137.81 (2x**C**H=C(CH_3)), 133.74 (two d's, CH_2=**C**H-CH_2-O, $^3J_{C-P}$ = 3.6 Hz, $^3J_{C-P}$ = 2.8 Hz), 119.04, 118.89 (**C**H=CH-CH_2-O), 111.93, 111.87 (2xNCH=**C**(CH_3)), 87.20 (two d's, C-4'A , $^3J_{4'A-P}$ = 3.6 Hz), 86.26 (two d's, C-4'B, $^3J_{3'B-P}$ = 2.8 Hz), 86.18 (C-1'A), 86.11 (C-1'B), 81.00 (two d's, C-3'A, $^2J_{3'A-P}$ = 4.5 Hz, $^2J_{3'A-P}$ = 4.7 Hz), 72.13, 71.96 (C-3'B), 70.23 (two d's, CH_2=CH-**C**H_2-O, $^2J_{C-P}$ = 4.5 Hz), 68.84 (d, C-5'B, $^2J_{5'B-P}$ = 6.4 Hz), 62.74, 62.70 (C-5'A), 40.88, 40.78 (C-2'B), 39.52 (d, C-2'A, $^3J_{2'A-P}$ = 3.6 Hz), 12.66, 12.46 (2xCH=**C**(CH_3)).

Ammonium phosphorothioate 4-9S

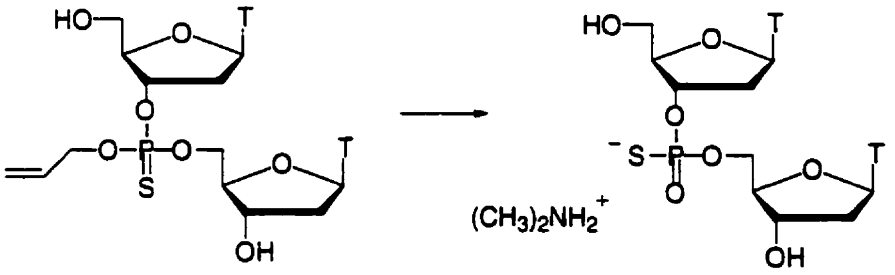


4-6S **4-9S**

A solution of the allyl protected **4-6S** (20 mg, 0.033 mmol) in concentrated aqueous ammonia (5 ml) was heated at 55 °C for 2 hours. Water was then removed by high vacuum and the product **4-9S** (18.7 mg, 97%) was obtained as a white solid.

^{31}P NMR (202.3 MHz, D_2O) δ 55.95, 55.58; ^{1}H NMR (500 MHz, CD_3OD) δ 7.91, 7.87 (two q's, 1H, C**H**=C(CH_3), $^{4}J_{H\text{-}H}$ = 1.0 Hz), 7.85, 7.84 (two q's, 1H, C**H**=C(CH_3), $^{4}J_{H\text{-}H}$ = 1.0 Hz), 6.35 (m, 1H, H-1'B), 6.28 (m, 1H, H-1'A), 5.06 (m, 1H, H-3'A), 4.52 (m, 1H, H-3'B), 4.03-4.22 (m, 4H, H-4'A, H-4'B, H-5'B), 3.81 (m, 2H, H-5'A), 2.43-2.50 (m, 1H, H-2'A), 2.16-2.31 (m, 3H, H-2'A, H-2'B), 1.97 (two d's, 3H, CH=C(C**H**$_3$), $^{4}J_{H\text{-}H}$ = 1.0 Hz), 1.87 (two d's, 3H, CH=C(C**H**$_3$), $^{4}J_{H\text{-}H}$ = 1.0 Hz); ^{13}C NMR (125.7 MHz, CD_3OD) δ 166.82, 166.80 (2xNHCOC(CH_3)), 152.74, 152.61 (2xNCONH), 138.18, 138.16 (2xCH=C(CH_3)), 112.11, 111.58 (2xNCH=C(CH_3)), 87.80 (two d's, C-4'A, $^{3}J_{4'A\text{-}P}$ = 5.5 Hz, $^{3}J_{4'A\text{-}P}$ = 6.4 Hz), 87.52 (two d's, C-4'B, $^{3}J_{4'B\text{-}P}$ = 8.3 Hz), 86.34, 86.30 (C-1'A), 86.20, 86.16 (C-1'B), 77.60 (d, C-3'A, $^{2}J_{3'A\text{-}P}$ = 5.5 Hz), 77.13 (d, C-3'A, $^{2}J_{3'A\text{-}P}$ = 4.5 Hz), 73.04, 72.99 (C-3'B), 66.59 (two d's, $C_{5'B}$, $^{2}H_{5'B\text{-}P}$ = 5.5 Hz), 62.92 ($C_{5'A}$), 40.99, 40.93 ($C_{2'B}$), 40.04 (two d's, $C_{2'A}$, $^{3}J_{2'A\text{-}P}$ = 3.6 Hz), 12.72, 12.51 (2xCH=C(CH_3)); MS (FAB-, NBA) m/z 561((dTP(S)(O)dT)$^{-1}$).

Dimethylammonium phosphorothioate 4-9S

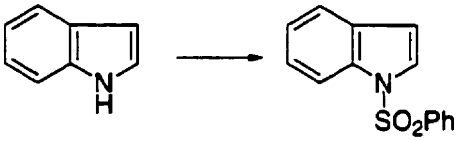


4-6S **4-9S**

A solution of the allyl protected **4-6S** (20 mg, 0.033 mmol) in 40% aqueous dimethylamine (5 ml) was stirred at room temperature for half an hour. Water was then removed under high vacuum to afford the product **4-9S** (18.6 mg, 93%) as a white solid. ^{31}P NMR (202 MHz, D_2O) δ 55.95, 55.58; ^1H NMR (500 MHz, CD_3OD) δ 7.90, 7.86 (two q's, 1H, C**H**=C(CH_3), $^4J_{H\text{-}H}$ = 1.0 Hz), 7.85 (two q's, 1H, C**H**=C(CH_3), $^4J_{H\text{-}H}$ = 1.0 Hz), 6.34 (m, 1H, H-1'B), 6.28 (m, 1H, H-1'A), 5.06 (m, 1H, H-3'A), 4.51 (m, 1H, H-3'B), 4.04-4.22 (m, 4H, H-4'A, H-4'B, H-5'B), 3.81 (m, 2H, H-5'A), 2.69 (s, 6H, $N(CH_3)_2$), 2.45 (m, 1H, H-2'A), 2.16-2.31 (m, 3H, H-2'A, H-2'B), 1.96 (two d's, 3H, CH=C(C**H**$_3$), $^4J_{H\text{-}H}$ = 1.0 Hz), 1.87 (two d's, 3H, CH=C(C**H**$_3$), $^4J_{H\text{-}H}$ = 1.0 Hz); MS (FAB-, NBA) m/z 561((dTP(S)(O)dT)$^{-1}$).

7.5. Experimentals for Chapter 5

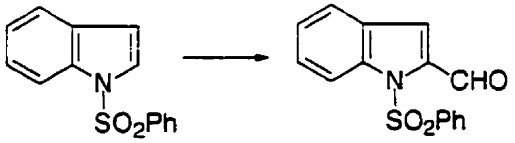
1-(Phenylsulfonyl)indole 5-1



5-1

To a suspension of sodium hydride (0.44 g, 11 mmol) in dry THF (10 ml) under argon at 0 °C was added slowly a solution of indole (1.18 g, 10 mmol) in THF (5 ml). The resulting mixture was stirred at room temperature for half an hour. Then the mixture was re-cooled to 0 °C, phenylsulfonyl chloride (1.4 ml, 11 mmol) was added dropwise over a period of 10 minutes. The mixture was warmed slowly to room temperature and stirred for 8 hours. Saturated sodium bicarbonate solution was added to the mixture. The aqueous layer was extracted with ethyl acetate. The extracts were washed with water and brine, and dried over magnesium sulfate. The solvent was removed to provide a pale yellow oil, which was dissolved in mixture of hexanes and ether (v/v=2:1) at room temperature and then left in the fridge. Crystals formed from the solution to afford the desired product **5-1** (2.32 g, 90%): m.p. 77-78 °C.

1-Phenylsulfonyl-indole-2-carboxylic aldehyde 5-2



5-1 **5-2**

To a solution of 2.0 M lithium diisopropylamide (LDA) (0.55 ml, 1.1 mmol) in dry THF (20 ml) at –78 °C under argon was added dropwise the solution of **5-1** (0.26 g, 1.0 mmol) in dry THF (20 ml). The mixture was stirred for 1.5 hours at –78 °C and then warmed up to 0 °C and stirred for 1 hour. The mixture was re-cooled to –78 °C and anhydrous DMF (0.16 ml, 2 mmol) was added slowly. The solution was then warmed slowly to room temperature and stirred for one additional hour. Saturated ammonium chloride was added to the reaction mixture. Extracted with dichloromethane several times, the combined organic extracts were washed with water and brine, and dried over magnesium sulfate. Purification by flash chromatography afforded **5-2** (1.65 g, 79%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.24 (d, 1H, J = 8.5 Hz), 7.78 (d, 2H, J = 7.5 Hz), 7.63 (d, 1H, 8.0 Hz), 7.52-7.54 (m, 2H), 7.48 (s, 1H), 7.42 (t, 2H), 7.32 (t, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 183.0, 138.33, 137.61, 137.40, 134.14, 129.20, 128.74, 128.02, 126.46, 124.74, 123.51, 118.96, 115.19.

N-trityl-imidazole 5-3

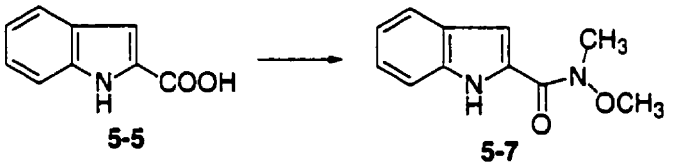
5-3

To a solution of trityl chloride (2.79 g, 10 mmol) in dry dichloromethane (50 ml) at 0 ° C was added slowly a solution of imidazole (0.68 g, 10 mmol) and triethylamine

(1.89 ml, 10 mmol) in dry dichloromethane (50 ml). The mixture was allowed to warm up to room temperature and stirred overnight. The reaction mixture was washed with saturated ammonium chloride, water and brine, respectively. The organic layer was dried over magnesium sulfate. The solvent was removed to furnish the crude **5-3**, which was recrystallized from dichloromethane / hexanes as a white solid (2.80 g, 90 %).

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43 (m, 1H, H-2), 7.3-7.4 (m, 9H, 3xC_6H_5), 7.1-7.2 (m, 6H, 3xC_6H_5), 7.0 (m, 1H, H-4), 6.81 (m, 1H, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.3, 139.0, 129.6, 128.3, 128.2, 121.6; MS (FAB, NBA) m/z 311 (1.8%, $(M+H)^+$); 243 (100%, $(C(C_6H_5)_3^+$).

Indole-2-Carboxylic acid Weinreb amide 5-7



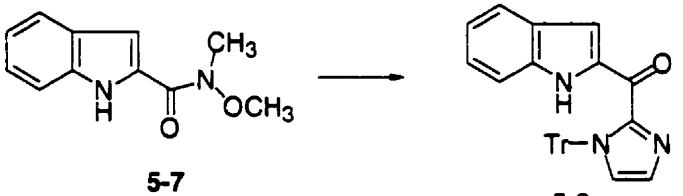
5-5 **5-7**

To a solution of indole-2-carboxylic acid **5-5** (0.645 g, 4 mmol) in anhydrous 1,2-dimethoxyethane (DME) (15 ml) was added triethylamine (0.8 ml, 5.7 mmol). Thionyl chloride (0.58 ml, 8 mmol) was then added very slowly at 0 °C over a period of 10 minutes. The reaction mixture was stirred at 0 °C for half an hour. The precipitate was filtered off. The filtrate was concentrated *in vacuo* to give the crude indole-2-carboxylic acid chloride **5-6** as a dark brown solid. The crude **5-6** and *N,O*-dimethylhydroxylamine hydrochloride (0.39 g, 4 mmol) were dissolved in dry dichloromethane (10 ml). The

solution was cooled down to 0 °C and pyridine (0.71 ml, 8.8 mmol) was added. The mixture was stirred at ambient temperature for 1 hour and evaporated *in vacuo*. The residue was partitioned between brine and a 1:1 mixture of dichloromethane and ether, and the organic layer was dried over magnesium sulfate. Purification by flash chromatography gave the desired Weinreb amide **5-6** (0.60 g, 73 % from acid) as a bright yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.78 (br, 1H, NH), 7.71 (d, 1H, H-4, J_{4-5} = 8.0 Hz), 7.46 (d, 1H, H-7, J_{7-6} = 7.5 Hz), 7.31 (m, 1H, H-6), 7.26 (s, 1H, H-3), 7.15 (m, 1H, H-5), 3.82 (s, 3H, OCH_3), 3.42 (s, 3H, NCH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 161.52, 135.74, 128.08, 127.81, 124.66, 122.36, 120.23, 111.67, 107.81, 61.18, 33.11; MS (CI, NH_3) m/z 205 ($(M+1)^+$, 60.4%), 174 (35.3%), 144 (100%).

2-Indolyl-N-trityl-2-imidazolyl ketone 5-8



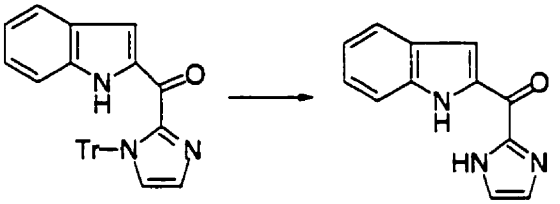
5-7 **5-8**

n-Butyllithium solution in hexane (1.6 M) (1.37 ml, 2.2 mmol) was added to a stirred solution of N-trityl imidazole **5-3** (0.62 g, 2.0 mmol) in dry THF (20 ml) at -78 °C under argon. The resulting solution was stirred at -78 °C for half an hour and allowed to warm up slowly to room temperature and stand for an hour. The original colorless solution turned to red gradually. The mixture was then re-cooled to -78 °C and indole-2-Weinreb amide **5-7** (0.204 g, 1 mmol) in dry THF (10 ml) was added. The mixture was

allowed to warm up to room temperature and stirred for an additional hour. The reaction mixture was then poured into a mixture of 5% hydrochloric acid and ether at 0 °C. The mixture was partitioned between brine and a mixture of ether and dichloromethane (1:1). Purification by flash chromatography afforded the desired product **5-8** (0.28 g, 62%) as a yellow solid and recovered Weinreb amide **5-7** (0.07 g, 35 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.50 (br, 1H, NH), 7.62 (d, 1H, H-4, J_{4-5} = 8.0 Hz), 7.35 (m, 2H, H-7 and H of imidazole), 7.24-7.29 (m, 11H, 9 H of trityl, H-6, H of imidazole), 7.12-7.18 (m, 7H, 6H of trityl, H-3), 7.06 (m, 1H, H-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.80, 144.91, 142.67, 137.10, 135.68, 129.81, 127.61, 127.58, 127.32, 127.27, 127.21, 125.52, 122.97, 120.36, 112.10, 111.34, 78.08; MS (FAB, NBA) m/z 454 $(M+1)^+$.

2-Indolyl-2-imidazolyl ketone 5-9



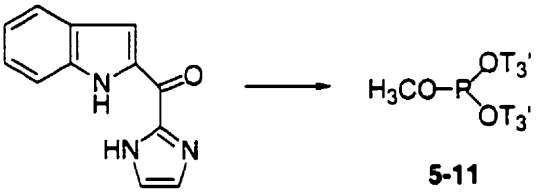
5-8 **5-9**

2-Indolyl-N-trityl-2-imidazolyl ketone **5-8** (0.44 g, 1 mmol) was dissolved in 5% acetic acid / methanol (10 ml). The mixture was brought to reflux for half an hour. The solvent was removed *in vacuo* and the residue was taken up in ethyl acetate, washed with saturated sodium bicarbonate solution, distilled water and brine respectively, and dried

over magnesium sulfate. Purification by flash chromatography yielded the product as a yellow solid (0.20 g, 97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 12.46 (br, 1H, NH of imidazole), 11.47 (br, 1H, NH of indole), 8.01 (s, 1H, H of imidazole), 7.76 (m, 1H, H-4), 7.65 (m, 1H, H-7), 7.52 (s, 1H, H of imidazole), 7.36 (s, 1H, H-3), 7.33 (m, 1H, H-6), 7.12 (m, 1H, H-5); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 172.74, 146.26, 138.99, 135.22, 131.79, 128.45, 126.61, 123.75, 121.72, 121.36, 113.64, 112.57; HRMS (EI) $C_{12}H_9N_3O$ calc: 211.0746, found: 211.0748.

Phosphite triester 5-11



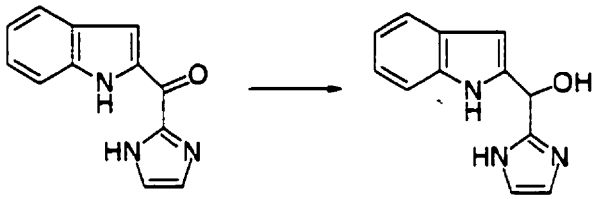
5-9 **5-11**

To a solution of methyl dichlorophosphite (7.4 µl, 0.078 mmol) in dry dichloromethane (0.2 ml) in a scrupulously dried NMR tube at 0 °C was added slowly a solution of **5-9** (15 mg, 0.07 mmol) in dry dichloromethane (0.6 ml) containing triethylamine (21.7 µl, 0.156 mmol). The NMR tube was then sealed. When ^{31}P NMR showed the disappearance of starting material and the formation of a new resonance at 80 ppm assigned to **5-10**, 5'-O-TBDMS thymidine (30 mg, 0.084 mmol) in dry dichloromethane was added. The NMR tube was allowed to stand at room temperature

for 15 hours, the crude mixture was purified by flash chromatography to afford the product **5-11** as a white solid (26.1 mg, 81%).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 140.84; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (br, 1H, NH), 9.19 (br, 1H, NH), 7.45 (2s's, 2H, 2XH-6), 6.36 (m, 2H, 2XH-1'), 4.79-4.89 (m, 2H, 2XH-3'), 4.10 (m, 2H, 2XH-4'), 3.79-3.90 (m, 4H, 2XH-5'), 3.56 (d, 3H, OCH_3), 2.42-2.52 (m, 2H, H-2'), 2.11 (m, 2H, H-2'), 1.89 (2 s's, 6H, 2XC=CMe), 0.93 (2 s's, 18H, $2XSiC(CH_3)_3$), 0.12 (2 s's, 12H, $2XSi(CH_3)_2$); MS (FAB, NBA/NaCl) m/z 795 (M+Na-1).

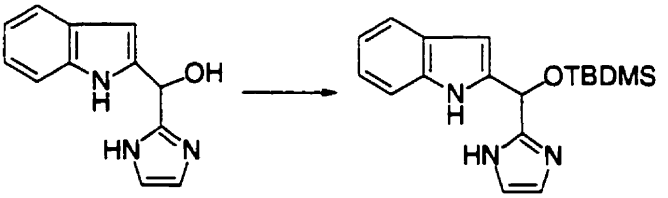
2-Indolyl-2-imidazolyl carbinol 5-12



5-9 **5-12**

2-Indolyl-2-imidazolyl ketone **5-9** (0.21 g, 1 mmol) in dry THF was added slowly into a suspension of lithium aluminum hydride (37.9 mg, 1 mmol) in THF (5 ml) at 0 °C, and the mixture was allowed to stir at room temperature for 10 minutes. A few drops of ethyl acetate were added to the reaction mixture to destroy the excess lithium aluminum hydride. Standard work-up gave alcohol **5-12**, which was used in the next step without further purification.

Silyloxy ether 5-13

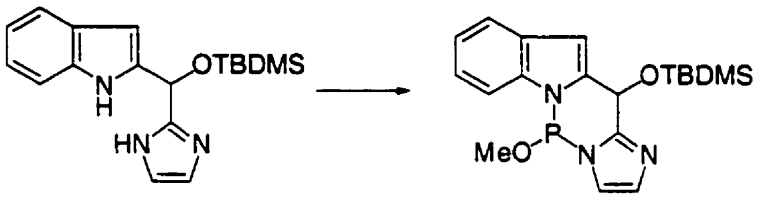


5-12 **5-13**

tert-Butyldimethylsilyl chloride (0.18 g, 1.2 mmol) was added to a solution of **5-12** (0.213 g, 1mmol) in DMF (10 ml) containing imidazole (0.136 g, 2.0 mmol) at room temperature. The solution was stirred for 2 hours. The solvent was removed under high vacuum, the residue was dissolved in ethyl acetate, washed with water and brine respectively. Purification by flash chromatography provided the desired product as a light yellow solid (0.31 g, 95 % from **5-9**).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.38 (br, 1H, NH of imidazole), 8.90 (br, 1H, NH of indole), 7.57 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.29 (d, 1H, H-7, J_{6-7} = 8.0 Hz), 7.13 (m, 1H, H-6), 7.07 (m, 1H, H-5), 7.03 (s, 1H, H of imidazole), 6.96 (s, 1H, H of imidazole), 6.46 (s, 1H, H-3), 6.18 (s, 1H, HCOTBDMS), 0.97 (s, 9H, $SiC(CH_3)_3$), 0.14 (s, 3H, $Si(CH_3)$), 0.07 (s, 3H, $Si(CH_3)$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 148.48, 138.34, 136.07, 128.21, 127.95, 121.70, 120.38, 119.59, 115.40, 110.92, 99.22, 66.63, 25.65, -5.23, -5.46; HRMS (EI) $C_{18}H_{25}N_3OSi$ calc: 327.1767, found: 327.1770.

Cyclic phosphite monoester 5-14



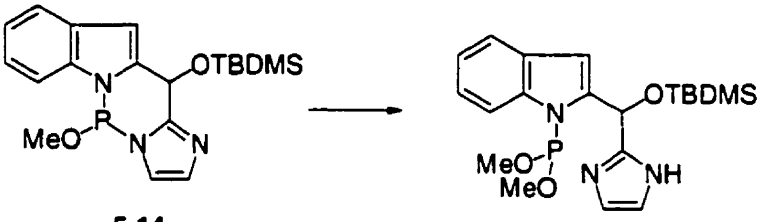
5-13 **5-14**

To a solution of methyl dichlorophosphite (6.35 µl, 0.067 mmol) in dry dichloromethane (0.2 ml) in a scrupulously dried NMR tube at 0 °C was added slowly a solution of **5-13** (20 mg, 0.06 mmol) in dry dichloromethane (0.6 ml) containing triethylamine (18.4 µl, 0.132 mmol). The NMR tube was then sealed and the reaction was followed by ^{31}P NMR. After 24 hours at 50 °C, the crude mixture was purified by flash chromatography to afford compound **5-14** as a white solid (16.3 mg, 70 %).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 80.47; ^{1}H NMR (500 MHz, $CDCl_3$) 7.78 (d, 1H, H-4, J_{4-5} = 8.0 Hz), 7.64 (d, 1H, H-7, J_{6-7} = 7.5 Hz), 7.33 (m, 1H, H-5), 7.24-7.27 (m, 2H, H-6 and H of imidazole), 7.21 (m, 1H, H of imidazole), 6.73 (s, 1H, H-3), 6.06 (s, 1H, HCOTBDMS), 3.52 (d, 3H, OCH_3, $^{3}J_{H-P}$ = 9.0 Hz), 0.80 (s, 9H, $SiC(CH_3)_3$), 0.26 (s, 3H, $Si(CH_3)$), 0.19 (s, 3H, $Si(CH_3)$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 147.27 (d, C-2 of imidazole, $^{3}J_{P-C}$ = 5.5 Hz), 140.17 (d, C-2, $^{2}J_{P-C}$ = 24.7 Hz), 139.43 (d, C adjacent to N-1 of indole, $^{2}J_{C-P}$ = 7.3 Hz), 130.35 (d, C-5 of imidazole, $^{2}J_{C-P}$ = 8.24 Hz), 129.49 (d, C between C-3 and C-4 of indole, $^{3}J_{C-P}$ = 3.66 Hz), 123.60 (C-5), 122.00 (C-6), 121.46 (d, C-4 of imidazole, $^{3}J_{P-C}$ = 19.23 Hz), 121.17 (d, C-7, $^{3}J_{C-P}$ = 12.8 Hz), 110.89 (d, C-4, $^{4}J_{C-P}$ = 17.4 Hz), 107.74 (C-3), 61.45 (CHOTBDMS), 52.92 (d, OCH_3, $^{2}J_{P-C}$ = 8.13 Hz),

25.55 ($SiC(CH_3)_3$), 18.05 ($SiC(CH_3)_3$), -4.91 ($SiCH_3$), -5.33 ($SiCH_3$); MS (FAB) m/z 388 $(M+H)^+$.

Dimethyl indolyl phosphite 5-15



5-14 **5-15**

Methanol (0.10 ml) was added to a mixture containing **5-14** obtained from the previous experiment in a NMR tube. Within 30 minutes, ^{31}P NMR indicated the completion of the reaction. The crude mixture was purified by flash chromatography to afford compound **5-15** as a white solid (16.4 mg, 65 % from **5-13**).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 147.50; ^1H NMR (500 MHz, $CDCl_3$) δ 9.18 (br, 1H, NH of imidazole), 8.67 (br, 1H, NH of indole), 7.89 (d, 1H, H-4, J_{4-5} = 8.0 Hz), 7.53 (d, 1H, H-7, J_{7-6} = 7.5 Hz), 7.09-7.15 (m, 2H, H-5, H-6), 6.96 (m, 2H, 2H of imidazole), 6.68 (s, 1H, H-3), 6.35 (s, 1H, CH(OTBDMS)), 3.45 (d, 3H, OCH_3, J_{H-P} = 12.5 Hz), 3.28 (d, 3H, OCH_3, J_{H-P} = 13.0 Hz), 0.93 (s, 9H, $SiC(CH_3)_3$), 0.062 (s, 3H, $Si(CH_3)$), 0.036 (s, 3H, $Si(CH_3)$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 148.64, 142.59, 142.45, 138.54, 138.51, 129.76, 128.92, 123.74, 121.29, 120.71, 115.56 (d, $^4J_{C-P}$ = 2.77 Hz), 115.02, 106.03 (d, $^3J_{C-P}$ = 3.77 Hz), 99.39, 52.28 (d, $^2J_{C-P}$ = 17.5 Hz), 51.65 (d, $^2J_{C-P}$ = 18.4 Hz), 25.80, -4.91, -5.33.

Phosphorothioate 5-16

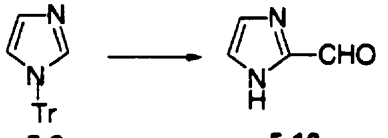
5-15 **5-16**

To a solution of **5-15** (20 mg, 0.06 mmol) in dry dichloromethane (0.25 ml) in a NMR tube at room temperature was added a solution of 5'-O-TBDMS-thymidine (42 mg, 0.12 mmol) in dry dichloromethane (0.45 ml) containing DBU (45 μl, 0.30 mmol). The NMR tube was then sealed. ^{31}P NMR indicated the reaction went to completion within 20 minutes. The mixture was then loaded to the top of column to run a fast column to filter off DBU (acetonitrile:dichloromethane = 1:1). The solvent was removed *in vacuo* and the residue was re-dissolved in dichloromethane and Beaucage's sulfurizing reagent (24 mg, 0.12 mmol) was added. After 10 minutes, the mixture was diluted with dichloromethane, washed with brine and dried over magnesium sulfate. Purification by flash chromatography provided the desired product **5-16** as a white solid (23.6 mg, 82%) and recovered **5-13** (15.6 mg, 80%)

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 70.98; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (br, 1H, NH), 7.51 (s, 1H, H-6), 6.38 (dd, 1H, H-1', $J_{1'-2'}$ = 9.0 Hz, 5.5 Hz), 5.11 (m, 1H, H-3'), 4.26 (m, 1H, H-4'), 3.90 (m, 2H, H-5'), 3.76 (2 d's, 6H, $2XOCH_3$, J_{H-P} = 13.5 Hz), 2.50 (m, 1H, H-2'), 2.11 (m, 1H, H-2'), 1.92 (s, 3H, C=CMe), 0.94 (9H, $SiC(CH_3)_3$), 0.14 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.21, 149.99, 135.02, 111.21, 85.81

(d, $J_{C\text{-}P}$ = 4.5 Hz), 84.65, 79.29, 63.39, 54.74, 39.22 (d, $J_{C\text{-}P}$ = 5.53 Hz), 29.70, 25.76, 18.18, 12.33, -5.56, -5.59; MS (ES) m/z = 479 (M-H), 959 (M+M-H).

Imidazole-2-carboxyaldehyde 5-18

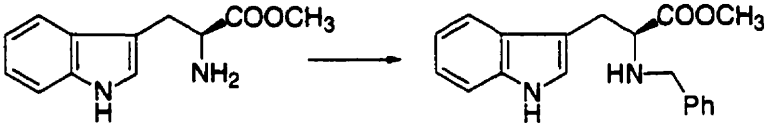


5-3 **5-18**

To a solution of N-trityl-imidazole **5-3** (1.55 g, 5 mmol) in dry THF (50 ml) at -78 °C under argon was added slowly a 1.6 M solution of *n*-Butyllithium in hexanes (3.44 ml, 5.5 mmol). The resulting dark red solution was stirred at -78 ° C for half an hour, and DMF (0.77 ml, 10 mmol) was added to the mixture. The reaction mixture was allowed to warm up to room temperature with stirring for another an hour. The solvent was removed *in vacuo*. The residue was taken up in ethyl acetate, washed with saturated ammonium chloride, water and brine, and dried over magnesium sulfate. The solvent was evaporated to give crude N-trityl-imidazole-2-carboxyaldehyde as a white solid. To the crude aldehyde was added 5% acetic acid in methanol (50 ml), the mixture was brought to reflux for an hour. The reaction mixture was concentrated *in vacuo*. Distilled water (50 ml) was added to the residue, producing white precipitate. The mixture was cooled down to 0 °C and filtered. The white precipitate was washed with cold distilled water (10 ml). The filtrate was evaporated *in vacuo*. the residue was purified by column chromatography to yield the desired product **5-18** as a white solid (0.39 g, 82 % for two steps).

^{1}H NMR (500 MHz, CD_3OD) δ 9.67 (s, 1H, CHO); 7.41 (s, 2H, H-4 and H-5 of imidazole); MS (EI) m/z 96 (100%, M^+).

N_b-benzyl-L-tryptophan methyl ester 5-19



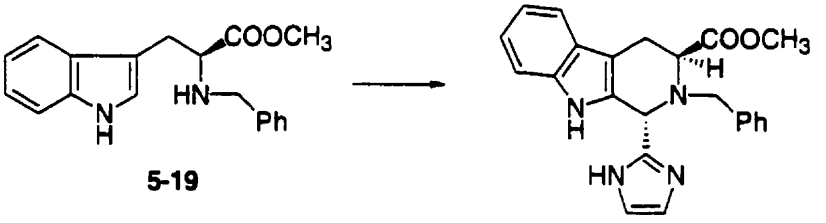
5-17 **5-19**

To a solution of L-tryptophan methyl ester **5-17** (0.218 g, 1 mmol) and benzaldehyde (0.11 ml, 1.1 mmol) in methanol (10 ml) was added sodium cyanoborohydride (62.8 mg, 1 mmol). The pH of the solution was then adjusted to around 6 by adding acetic acid. The mixture was stirred at room temperature for 3 hours, and acetic acid was added during the reaction occasionally to maintain the pH of the solution at 6. Distilled water was added to the reaction mixture till the white cloudy precipitate was formed, pH of the mixture was then adjusted to around 8 by adding aqueous ammonia. The mixture was extracted with chloroform several times, the combined organic extracts were washed with brine, and dried over magnesium sulfate. Purification by flash chromatography afforded the product **5-19** as a white solid (0.25g, 80%).

^{1}H NMR (500MHz, $CDCl_3$) δ 8.21 (br, 1H, NH of indole), 7.60 (d, 1H, H-4, J_{4-5} = 8.0 Hz), 7.32 (d, 1H, H-7, J_{7-6} = 8.5 Hz), 7.24-7.30 (m, 5H, phenyl protons), 7.20 (m, 1H, H-6), 7.13 (m, 1H, H-5), 6.98 (d, 1H, H-2, J_{2-NH} = 2.0 Hz), 3.86 (d, 1H, 1H of CH_2Ph, J = 13.5 Hz), 3.65-3.72 (m, 5H, 1H of CH_2Ph, $CHCOOCH_3$, $COOCH_3$), 3.20 (m, 2H,

$CH_2CHCOOMe$), 1.95 (br, 1H, NH); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 175.31, 139.59, 136.10, 128.28, 128.09, 127.41, 126.94, 122.81, 121.95, 119.32, 118.71, 111.10, 111.07, 61.17, 52.06, 51.68, 29.24; MS (FAB, NBA) m/z 309.08 (100%, $(M+H)^+$).

2-Benzyl-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-2-imidazole 5-20



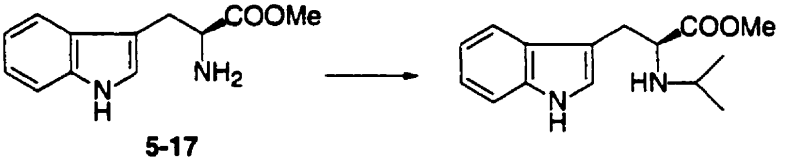
5-19 **5-20**

N_b-Benzyl-L-tryptophan methyl ester **5-19** (0.31 g, 1 mmol), imidazole-2-carboxylic aldehyde **5-18** (0.096 g, 1 mmol) and *p*-toluene sulfonic acid (0.038 g, 0.2 mmol) were dissolved in benzene (50 ml). The mixture was then brought to reflux using Dean-Stark water trap to remove water. After 18 hours, the solvent was removed *in vacuo*. The residue was purified by flash chromatography to furnish the desired product **5-20** as a yellow solid (0.25 g, 65%).

^1H NMR (500 MHz, CD_2Cl_2) δ 9.91 (br, 1H, NH of indole), 7.50 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.28-7.36 (m, 5H, H of phenyl), 7.22 (d, 1H, H-7, J_{7-6} = 7.0 Hz), 7.01-7.10 (m, 2H, H-5, H-6), 6.93 (s, 2H, H of imidazole), 5.60 (s, 1H, (NH)N=CC**H**N(Bn)), 3.98-4.02 (m, 2H, C**H**$COOCH_3$, 1H of CH_2Ph), 3.86 (d, 1H, 1H of CH_2Ph, J = 14.0 Hz), 3.70 (s, 3H, $COOCH_3$), 3.21 (m, 2H, C$H_2$$CHCOOCH_3$); ^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 173.24,

148.67, 139.00, 137.25, 131.98, 128.89, 127.74, 126.98, 121.95, 119.39, 118.35, 111.60, 106.04, 60.61, 57.76, 55.94, 52.02, 51.20, 23.54, 21.18, 14.35; HRMS (EI) $C_{23}H_{22}N_4O_2$ calc: 386.1743, found: 386.1745.

N_b-isopropyl-L-tryptophan methyl ester



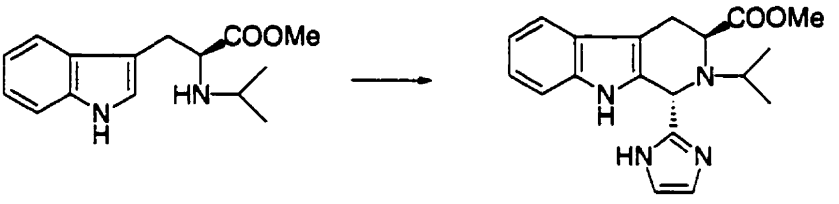
5-17

To a solution of L-tryptophan methyl ester **5-17** (0.218 g, 1 mmol) and acetone (0.15 ml, 2 mmol) in methanol (10 ml) was added sodium cyanoborohydride (62.8 mg, 1 mmol), the pH of the solution was then adjusted to around 6 by adding acetic acid. The resulting mixture was stirred at room temperature for 3 hours. Acetic acid was added during the reaction to maintain the pH of the solution at 6. Distilled water was added to the reaction mixture till the white cloudy precipitate was formed. The pH of the mixture was adjusted to around 8 by adding aqueous ammonia. The mixture was extracted with chloroform several times, the combined organic extracts were washed with brine, and dried over magnesium sulfate. The solvent was removed to provide the product as a white solid (0.24 g, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (br, 1H, NH of indole), 7.59 (d, 1H, H-4 of indole), 7.35 (d, 1H, H-7 of indole), 7.19 (m, 1H, H-6 of indole), 7.12 (m, 1H, H-5 of indole), 7.09 (s, 1H, H-2 of indole), 3.76 (m, 1H, C**H**COOMe), 3.60 (s, 3H, OCH_3), 3.16 (d, 2H, C**H$_2$**CHCOOMe), 2.78 (hepta, 1H, C**H**$(CH_3)_2$), 1.06 (d, 3H, CH_3 of isopropyl), 1.01 (d,

3H, CH_3 of isopropyl); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 161.91 (**C**OOMe), 136.13, 127.42, 122.78 (C-2), 122.05 (C-6), 119.42 (C-5), 118.70 (C-4), 111.11 (C-7), 59.64 (**C**HCOOMe), 51.71(OCH_3), 47.36 (**C**H(CH_3)$_2$), 29.48 (**C**H_2CHCOOMe), 23.50, 21.91 (2 **C**'s of isopropyl); MS (FAB, NBA) m/z 261 (100%, $(M+H)^+$).

2-Isopropyl-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-2-imidazole 5-22



5-22

N_b-isopropyl auxiliary **5-22** was prepared using the same procedure as described for the preparation of **5-20**.

^1H NMR (500 MHz, CD_2Cl_2) δ 11.62 (br, 1H, NH of imidazole), 9.90 (br, 1H, NH of indole), 7.47 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.30 (d, 1H, H-7, J_{7-6} = 8.0 Hz), 6.98-7.09 (m, 4H, H-5, H-6, 2H of imidazole), 5.37 (s, 1H, (NH)N=CC**H**N(*i*-Pr)), 4.26 (dd, 1H, CH_2C**H**$COOCH_3$, J = 6.0 Hz and 1.5 Hz), 3.63 (s, 3H, $COOCH_3$), 3.51 (d, 1H, CH_2CH$COOCH_3$, J = 15.0 Hz), 3.24 (m, 1H, C**H**(CH_3)$_2$), 3.00 (ddd, 1H, CH_2CH$COOCH_3$, J = 15.0 Hz, 6.6 Hz and 2.0 Hz), 1.18 (d, 3H, CH(C**H**$_3$)$_2$, J = 6.5 Hz), 1.07 (d, 3H, CH(C**H**$_3$)$_2$, J = 6.5 Hz); ^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 177.88, 150.76,

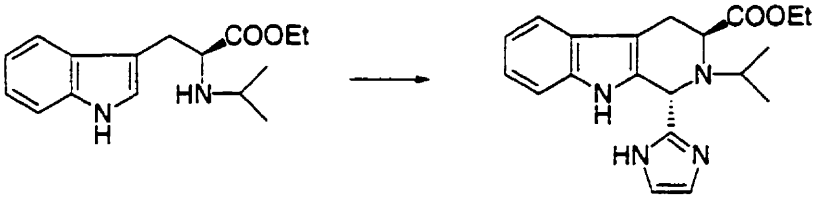
136.00, 131,59, 128.50, 126.60, 121.64, 119.13, 118.02, 115.53, 111.14, 104.82, 54.70, 54.57, 52.64, 52.25, 24.82, 20.93, 19.65; MS (EI) m/z 338 (M^+).

N_b-isopropyl-L-tryptophan ethyl ester

N_b-isopropyl-Ltryptophan ethyl ester was prepared using the same procedure as described for compound **5-19**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (br, 1H, NH of indole), 7.60 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.30 (d, 1H, H-7, J_{7-6} = 8.0 Hz), 7.16 (m, 1H, H-6), 7.10 (m, 1H, H-5), 7.00 (d, 1H, H-2, J = 2.0 Hz), 4.03 (q, 2H, $COOC\mathbf{H}_2CH_3$, J = 7.0 Hz), 3.71 (m, 1H, $CH_2C\mathbf{H}COOEt$), 3.11 (m, 2H, $C\mathbf{H}_2CHCOOEt$), 2.75 (m, 1H, $NC\mathbf{H}(i\text{-}Pr)_2$), 1.62 (br, 1H, $NC\mathbf{H}(i\text{-}Pr)$), 1.03 (d, 3H, $NCH(C\mathbf{H}_3)_2$, J = 6.5 Hz), 0.98 (d, 3H, $NCH(C\mathbf{H}_3)_2$, J = 6.0 Hz).

2-Isopropyl-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-2-imidazole 5-21



5-21

Ethyl ester **5-21** was prepared using the same procedure as described for the preparation of **5-20**.

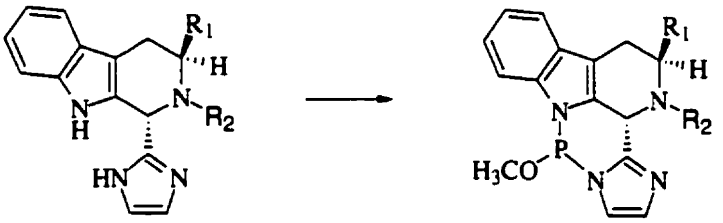
^{1}H NMR (500 MHz, CD_2Cl_2) δ 11.67 (br, 1H, NH of imidazole), 10.02 (br, 1H, NH of indole), 7.45 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.29 (d, 1H, H-7, J_{7-6} = 8.0 Hz), 6.95-7.07 (m, 4H, H-5, H-6, 2H of imidazole), 5.36 (s, 1H, (NH)N=CC**H**N(*i*-Pr)), 4.22 (m, 1H, CH_2**C**HCOOEt), 4.00-4.10 (m, 2H, COO**C**$H_2$$CH_3$), 3.50 (d, 1H, C$H_2$CHCOOEt, J = 15.0 Hz), 3.22 (m, 1H, C**H**$(CH_3)_2$), 2.98 (ddd, 1H, CH_2CHCOOEt, J = 15.0 Hz, 8.0 Hz and 1.5 Hz), 1.15 (m, 6H, CH(CH_3)$_2$, COOCH_2CH_3), 1.07 (d, 3H, CH(CH_3)$_2$, J = 7.0 Hz); MS (EI) m/z 352 (M^+).

2-Benzyl-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-2-imidazole 5-23

Decarbomethoxy **5-23** was prepared using the same procedure as described for the preparation of **5-20**.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 9.37 (br, 1H, NH of imidazole), 8.82 (br, 1H, NH of indole), 7.48 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.25 (d, 1H, H-7, J_{7-6} = 7.5 Hz), 7.05-7.12 (m, 2H, H-5, H-6), 6.94 (2 s's, 2H of imidazole), 5.22 (s, 1H, (NH)N=CC**H**N(*i*-Pr)), 3.19 (m, 1H, C$H_2$$CH_2$N(*i*-Pr), 2.94 (m, 1H, NC**H**$(CH_3)_2$), 2.73-2.83 (m, 3H, CH_2CH_2N(*i*-Pr)), 1.19 (d, 3H, NC**H**$(CH_3)_2$, J = 6.5 Hz), 1.04 (d, 3H, NC**H**$(CH_3)_2$, J = 6.5 Hz); ^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 149.04, 136.32, 132.48, 128.17, 126.57, 121.38, 118.70, 117.99, 115.44, 110.93, 108.15, 55.64, 50.57, 41.59, 21.63, 14.39; MS (EI) m/z 280 (100%, M^+).

Cyclic phosphite monoesters 5-24, 5-25, 5-26 and 5-27



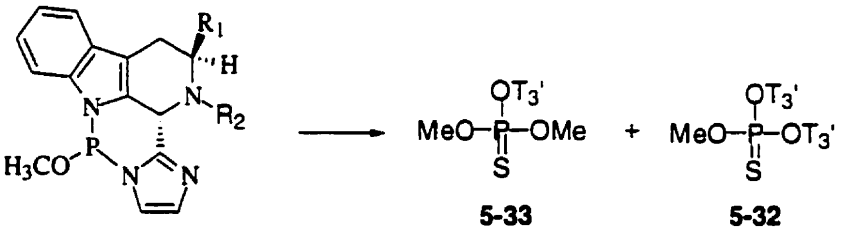
5-20: R_1 = COOMe, R_2 = Bn;
5-21: R_1 = COOEt, R_2 = *i*-Pr;
5-22: R_1 = COOMe, R_2 = *i*-Pr;
5-23: R_1 = H, R_2 = *i*-Pr

5-24: R_1 = COOMe, R_2 = Bn;
5-25: R_1 = COOEt, R_2 = *i*-Pr;
5-26: R_1 = COOMe, R_2 = *i*-Pr;
5-27: R_1 = H, R_2 = *i*-Pr

To a solution of methyl dichlorophosphite (4.1 µl, 0.044 mmol) in dry dichloromethane (0.45 ml) in a NMR tube at 0 °C was added slowly a solution of **5-20** (17.0 mg, 0.044 mmol) dissolved in dichloromathane (0.25 ml) containing triethylamine (12.8 µl, 0.092 mmol) under argon. The NMR tube was then sealed and allowed to stand at room temperature until ^{31}P NMR showed the disappearance of the resonance at 180 ppm and the formation of the new resonances at around 88 ppm and 91 ppm (10:1). The crude **5-24** was used in the next reaction without further purification.

Similarly, compounds **5-25**, **5-26** and **5-27** were prepared from **5-21**, **5-22** and **5-23**, respectively.

Phosphorothioates 5-32 and 5-33



5-33 **5-32**

5-24: R_1 = COOMe, R_2 = Bn;
5-25: R_1 = COOEt, R_2 = *i*-Pr;
5-26: R_1 = COOMe, R_2 = *i*-Pr;
5-27: R_1 = H, R_2 = *i*-Pr

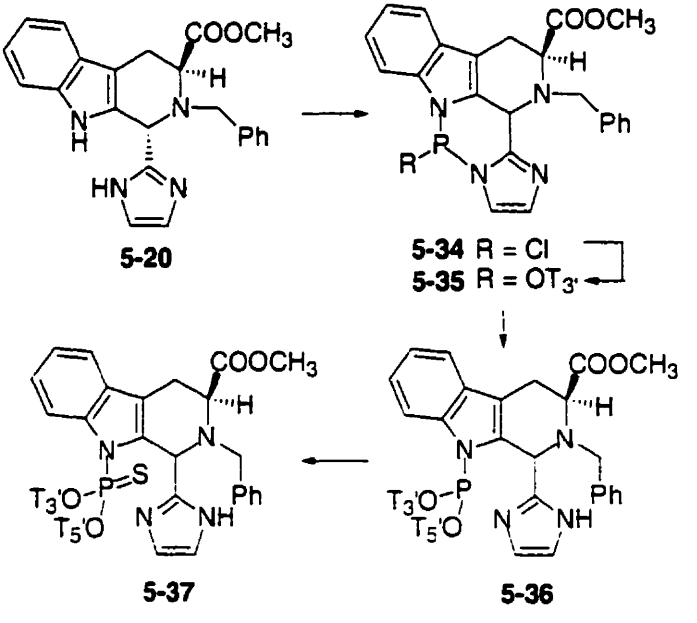
To the previous NMR tube containing **5-24** at room temperature was added 5'-O-TBDMS-thymidine (47 mg, 0.132 mmol) in dry dichoromethane (0.5 ml). The NMR tube was then sealed. ^{31}P NMR indicated the formation of a new resonance at 142.1 ppm immediately. Upon heating at 55 °C overnight, peaks at 142.1 ppm shifted to 140 ppm. Beaucage's reagent (10.6 mg, 0.053 mmol) was added to the reaction mixture. After 10 minutes, the reaction mixture was diluted with dichloromethane and washed with water and brine. Purification by flash chromatography afforded **5-32** (16 mg, 46.6%) and **5-33** (5 mg, 23.6%), both as white solids.

When **5-25**, **5-26** or **5-27** were used instead of **5-24**, similar results were obtained. Dithymidine phosphorothioate **5-32**: ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 68.03; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (br, 2H, NH of thymidines), 7.46 (2H, H-6 of thymidines), 6.36 (m, 2H, H-1'), 5.09 (m, 2H, H-3'), 4.24 (m, 2H, H-4'), 3.88 (m, 4H, H-5'), 3.76 (d, 3H, OCH_3, J_{P-H} = 13.5 Hz), 3.52 (m, 2H, H-2'), 2.11 (m, 2H, H-2'), 1.88 (s, 6H, 2xCH_3), 0.92 (2S's, 18 H, 2x$Si(C(CH_3)_3)$, 0.12 (12H, 2x$Si(CH_3)_2$); ^{13}C NMR (125.7 MHz, CD_2Cl_2) δ 163.62, 150.33, 134.92, 111.25, 85.68, 84.62 (d, J_{C-P} = 4.5 Hz), 79.67, 63.34,

54.80, 39.15 (d, $J_{C\text{-}P}$ = 4.7 Hz), 25.91, 25.62, 18.33, 12.52, -3.61, -5.39, -5.46; MS (CI) m/z 804 $(M+1)^+$.

Dimethoxy phosphorothioate **5-33**: ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 70.98; ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (br, 1H, NH), 7.51 (s, 1H, H-6), 6.38 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 9.0 Hz, 5.5 Hz), 5.11 (m, 1H, H-3'), 4.26 (m, 1H, H-4'), 3.90 (m, 2H, H-5'), 3.76 (2 d's, 6H, 2xOCH_3, $J_{H\text{-}P}$ = 13.5 Hz), 2.50 (m, 1H, H-2'), 2.11 (m, 1H, H-2'), 1.92 (s, 3H, C=CMe), 0.94 (9H, $SiC(CH_3)_3$), 0.14 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.21, 149.99, 135.02, 111.21, 85.81 (d, $J_{C\text{-}P}$ = 4.5 Hz), 84.65, 79.29, 63.39, 54.74, 39.22 (d, $J_{C\text{-}P}$ = 5.5 Hz), 29.70, 25.76, 18.18, 12.33, -5.56, -5.59; MS (ES) m/z = 479 (M-H), 959 (M+M-H).

Indolophosphorothioate 5-37

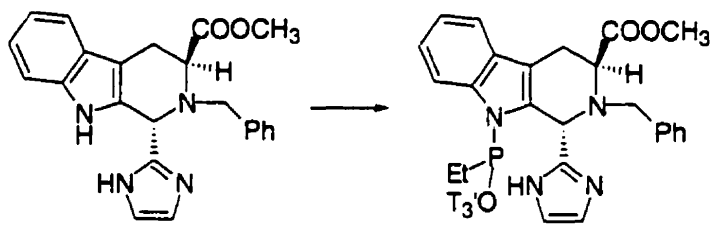


To a cooled solution of phosphorus trichloride (4.43 μl, 0.051 mmol) in dry dichloromethane in a NMR tube at 0 °C was added slowly a solution of **5-20** (19.6 mg, 0.051 mmol) in dichloromethane containing triethylamine (15.6 μl, 0.11 mmol). The NMR tube was sealed. ^{31}P NMR indicated the formation of peaks at 90.9 ppm and 89.6 ppm in a ratio of 1 to 11. A solution of 5'-O-TBDMS-thymidine (19 mg, 0.054 mmol) in dry dichloromethane (0.4 ml) containing triethylamine (7.9 μl, 0.056 mmol) was added slowly at 0 °C, the NMR tube was sealed and allowed to stand at room temperature. After ^{31}P NMR showing the formation of a major resonance at 87.0 ppm, a solution of 3'-O-TBDMS-thymidine (19.9 mg, 0.056 mmol) in dry dichloromethane (0.4 ml) was added to the reaction mixture at 0 °C. The NMR tube was sealed and warmed up slowly. ^{31}P NMR indicated the formation of resonance at 142 ppm within 10 minutes. Beaucage's sulfurizing reagent (12 mg, 0.061 mmol) was then added to the mixture, a major phosphorus resonance at 60 ppm was formed within 5 minutes. The reaction mixture was diluted with dichloromethane and washed with water and brine, and dried over magnesium sulfate. Purification by flash chromatography furnished the desired product **5-37** (25 mg, 42.3 %) as a white solid.

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 61.2 ppm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.77 (br, 1H, NH of imidazole), 8.69 (2 br's, 2H, NH of thymidines), 8.05 (d, 1H, H-4 of indole, J_{4-5} = 8.0 Hz), 7.55 (d, 1H, H-7 of indole, J_{7-6} = 7.5 Hz); 7.46 (2 s's, C=CH of thymidines); 7.21–7.36 (m, 7H, H-5, H-6 of indole and benzyl aromatic protons), 6.98 (s, 1H, H of imidazole), 6.77 (s, 1H, H of imidazole), 6.21 (t, 1H, H-1'), 5.51 (s, 1H, NCHimidazole), 5.42 (m, 1H, H-1'), 5.08 (m, 1H, H-3'), 4.17-4.22 (2H, H-3' and CH_2Ph), 4.02 (m, 1H, $CH_2CHCOOCH_3$), 3.51-4.04 (m, 10H, H-4' and H-5' of thymidines, CH_2Ph, $COOCH_3$),

3.08–3.16 (m, AB of ABX system, $CH_2CHCOOCH_3$), 1.98-2.17 (m, 3H, H–2'), 1.89 (2s's, 6H, $2XCH_3C{=}C$), 1.74 (m, 1H, H-2'), 0.81 (2s's, 18H, $2XSi(CH_3)_2C(CH_3)_3$), -0.03 (4s's, 12H, $2XSi(CH_3)_2C(CH_3)_3$; ^{13}C NMR (125.7MHz, $CDCl_3$) δ 172.49, 163.47, 150.13, 150.01, 147.55, 138.72, 137.85, 137.78, 135.56, 134.66, 132.69, 132.65, 129.03, 128.77, 127.52, 127.45, 123.94, 122.82, 118.94, 116.86, 116.18, 116.12, 114.73, 111.35, 111.22, 85.20 (d, C-4', $^3J_{C\text{-}P}$ = 5.4 Hz), 85.13, 84.82 (d, C-4'; $^3J_{C\text{-}P}$ = 10.1 Hz), 84.67, 79.61 (d, C-3', $^2J_{C\text{-}P}$ = 2.77 Hz), 71.85, 67.56 (d, $J_{C\text{-}P}$ = 7.29 Hz), 63.14, 55.87, 55.03, 52.26, 52.10, 40.48, 38.98 (PCH_2CH_3, $J_{C\text{-}P}$ = 1.1 Hz), 25.88, 25.80, 25.63, 20.33, 18.15, 17.79, 12.50, 12.48, -4.67, -4.88, -5.54, -5.56; HRMS (EI) $C_{55}H_{75}N_8O_{12}PSSi_2$ calc: 1158.4501, found: 1158.4505.

Alkyl phosphinate 5-41

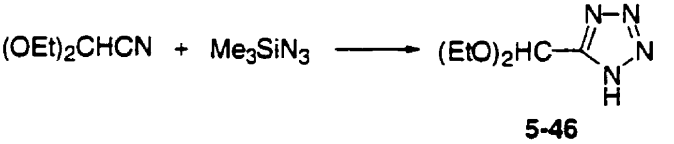


5-20 **5-41**

To a solution of dichloroethyl phosphine (12.5 µl, 0.11 mmol) in dry dichloromethane (0.20 ml) in a scrupulously dried NMR tube at –78 °C was added slowly a solution of **5-20** (44 mg, 0.11 mmol) in dry dichloromethane containing triethylamine (35 µl, 0.24 mmol). The NMR was then sealed. When ^{31}P NMR showed resonance at around 75 ppm, 5'-O-TBDMS thymidine (49 mg, 0.13 mmol) in dichloromethane (0.5 ml) was added to the NMR tube at 0 °C. The reaction mixture was allowed to warm up to room temperature and stand for half an hour. The reaction mixture was diluted with

dichloromethane, washed with water and brine. Purification by flash column chromatography afforded the desired product **5-41** (50 mg, 57%) as a white solid. ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 142.70; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.35 (br, 1H, NH of imidazole), 8.27 (br, 1H, NH of thymidine), 7.83 (d, 1H, H-4 of indole, J_{4-5} = 8.0 Hz), 7.52 (d, 1H, H-7 of indole, J_{7-6} = 7.5 Hz), 7.15–7.34 (m, 7H, H-5, H-6 of indole and benzyl aromatic protons), 6.95 (s, 1H, H of imidazole), 6.89 (s, 1H, H of imidazole), 6.09 (t, 1H, H-1'), 5.40 (s, 1H, NCHimidazole), 4.45 (m, 1H, H-3'), 4.08 (m, 1H, H-4'), 3.84-3.95 (m, 3H, H-5', $CH_2CHCOOCH_3$, CH_2Ph), 3.68-3.73 (m, 5H, H- 5', CH_2Ph, $COOCH_3$), 3.01–3.10 (m, AB of ABX system, $CH_2CHCOOCH_3$), 2.44-2.49 (m, 1H, H–2'), 2.00 (m, 1H, H-2'), 1.79–1.87 (m, 5H, CH_3C=C, PCH_2CH_3), 0.95–1.01 (m, 3H, PCH_2CH_3), 0.91 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.07 (s, 3H, $Si(CH_3)_2C(CH_3)_3$), 0.06 (s, 3H, $Si(CH_3)_2C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 172.64, 163.38, 149.84, 147.96, 138.94, 135.47, 129.95, 128.63, 128.51, 128.28, 127.50, 122.65, 120.97, 118.83, 115.90, 114.65, 110.70, 86.23 (d, C-4', $^{3}J_{C-P}$ = 8.29 Hz), 84.98, 79.47 (d, C-3', $^{2}J_{C-P}$ = 18.35 Hz), 63.27, 57.20, 55.17, 55.06, 53.14, 52.01, 38.81 (PCH_2CH_3, J_{C-P} = 4.53 Hz), 25.96, 24.22 (d, C-2', $^{3}J_{C-P}$ = 10.9 Hz), 21.39, 18.36, 7.50 (d, PCH_2CH_3, J_{C-P} = 19.2 Hz), -5.34, -5.37, HRMS (EI) $C_{41}H_{53}N_6O_7PSi$ calc: 800.3483, found: 800.3486.

Tetrazole-5-diethoxyacetal 5-46



5-46

A solution of diethoxyacetonitrile (0.28 ml, 2 mmol), azidotrimethylsilane (2.6 ml, 20 mmol) and pyridine (0.32 ml, 4 mmol) in chloroform (15 ml) was heated to 40 °C for two days. The solvent was removed, and water was added to the residue. The resulting solution was stirred for half an hour. The pH of the aqueous solution was adjusted to around 9–10, extracted with ether. Pyridine in the residue was azeotropically removed by adding toluene. The pH of the aqueous residue was then adjusted to 1.5-2, extracted with *n*-butanol with small amount of brine several times. The *n*–butanol extracts were collected and the solvent was removed *in vacuo*. The residue was taken up in chloroform. After removal of chloroform, the product was obtained as a white solid (0.176 g, 51%). The crude product **5-46** was used in the next reaction without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (s, 1H, C**H**$(OEt)_2$); 3.70 (m, 4H, CH($OC\mathbf{H}_2CH_3$)); 1.23 (t, 6H, CH($OCH_2C\mathbf{H}_3$)); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 162.00; 94.35; 62.91; 14.88; MS (CI) M/Z 173 (44%, M+1).

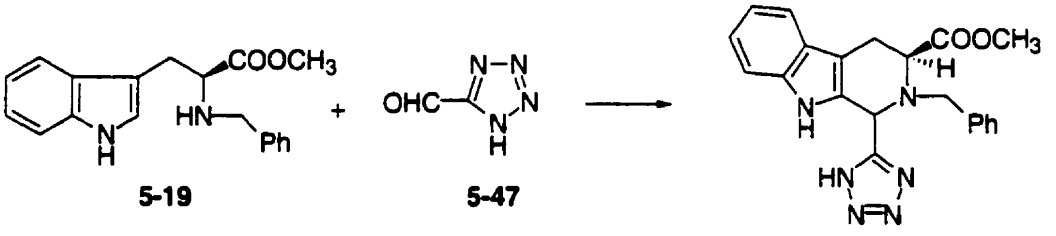
Tetrazole-5-aldehyde 5-47

5-46 **5-47**

Tetrazole acetal **5-46** (0.176 g, 1.02 mmol) obtained from the previous experiment was dissolved in a 4 N aqueous hydrochloric acid solution (30 ml). The resulting mixture was stirred at room temperature overnight. The solvent was then removed *in vacuo* to afford the product as a white solid in quantitative yield.

MS (EI) M/Z 98 (M^-, 40%).

2-Benzyl-3-(methoxycarbonyl)-1,2-3,4-tetrahydro-9H-pyrido[3,4-b]indole-2-tetrazole 5-48



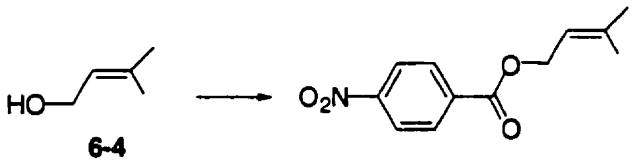
5-19 **5-47** **5-48**

N_b-Benzyl-L-tryptophan methyl ester **5-19** (0.31 g, 1 mmol), tetrazole-2-carboxylic aldehyde **5-47** (0.098 g, 1 mmol) and *p*-toluene sulfonic acid (0.038 g, 0.2 mmol) were dissolved in benzene (50 ml). The mixture was then brought to reflux using Dean-Stark water trap to remove water. After 18 hours, the solvent was removed *in vacuo*. The residue was purified by flash chromatography to furnish the desired product **5-48** as a yellow solid (0.19 g, 51%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (br, 1H), 7.18 (d, 1H, J = 8.0 Hz), 6.93-7.03 (m, 6H), 6.82-6.84 (t, 1H), 6.75-6.78 (t, 1H), 5.82 (s, 1H), 3.79 (d, 1H, J = 14.5 Hz), 3.69 (m, 1H), 3.55 (d, 1H, J = 14.0 Hz), 3.34 (s, 3H), 2.86-2.96 (d, 3H); ^{13}C NMR (125.7MHz, $CDCl_3$) δ 172.18, 137.41, 136.57, 129.09, 127.91, 126.86, 125.78, 121.57, 118.73, 118.10, 117.65, 110.94, 106.49, 56.05, 54.82, 51.68, 50.95, 30.12, 23.57; MS (FAB, NBA) m/z 389 ($(M+1)^+$, 43.6%).

7.6. Experimentals for Chapter 6

3,3-Dimethylallyl *p*-nitrobenzoate 6-6

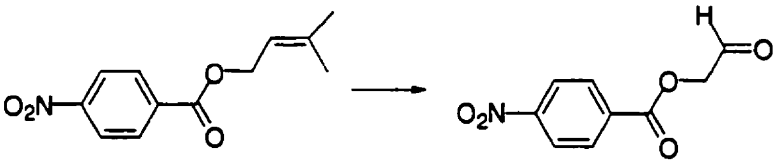


6-4 **6-6**

To a solution of 3-methyl-2-buten-1-ol **6-4** (1.02 ml, 10 mmol) in pyridine (20 ml) at 0 °C was added slowly 4-nitrobenzoyl chloride (1.85 g, 10 mmol), followed by 4-dimethyaminopyridine (DMAP) (0.12 g, 1 mmol). The resulting mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*, coevaporated with toluene several times. The residue was taken up in dichloromethane, washed with saturated ammonium chloride, water and brine, and dried over magnesium sulfate. Purification by flash chromatography afforded the desired product 3,3-dimethylallyl *p*-nitrobenzoate **6-6** as a white solid (2.1 g, 91%).

^{1}H NMR (500 MHz, CDCl3) δ 8.25 (d, 2H, aromatic protons, J = 8.5 Hz), 8.18 (d, 2H, aromatic protons, J = 8.5 Hz), 5.44 (t, 1H, $OCH_2\mathbf{C}H{=}C(CH_3)_2$, J = 7.0 Hz), 4.83 (d, 2H, $O\mathbf{CH_2}CH{=}C(CH_3)_2$, J = 7.0 Hz), 1.76 (d, 6H, $2XCH_3$, J = 8.5 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.70, 150.40, 140.15, 135.87, 130.67, 123.44, 117.92, 62.73, 25.78, 18.01; MS (CI) m/z 236 ($(M+1)^+$, 2.75%).

2-Oxoethyl *p*-nitrobenzoate 6-7

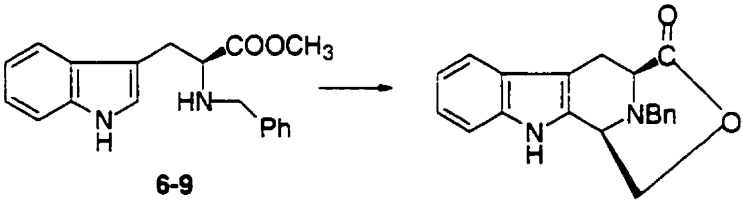


6-6 **6-7**

A solution of 3,3-dimethylallyl *p*-nitrobenzoate **6-6** (2.0 g, 8.5 mmol) in methanol was cooled down to –78 °C and ozone was bubbled till the color of the solution turned to blue. Dimethyl sulfide was then added at – 78 °C and the solution was allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the residue was taken up in chloroform, washed with water and dried over magnesium sulfate. Purification by flash chromatography afforded the desired product 2-oxoethyl *p*-nitrobenzoate **6-7** as a white solid (1.6 g, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.71 (s, 1H, CHO), 8.29 (d, 2H, aromatic protons, J = 8.0 Hz), 8.25 (d, 2H, aromatic protons, J = 8.0 Hz), 5.00 (s, 2H, C**H**$_2$CHO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 194.46, 163.97, 150.76, 134.15, 131.01, 123.61, 69.48; MS (CI) m/z 210 ($(M+1)^+$, 6.73%), 154 (100%).

Lactone 6-12



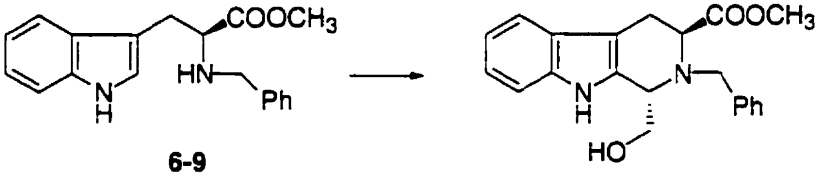
6-9 **6-12**

A mixture of N_b-benzyl-L-tryptophan methyl ester **6-9** (0.31 g, 1 mmol) and aldehyde **6-7** (0.23 g, 1.1 mmol) in toluene was brought to reflux with Dean-Stark water

trap to remove water. After 4 hours, the reaction was stopped. The solvent was removed to afford the crude condensation product, which was dissolved in 1N sodium methoxide in methanol (5 ml) at room temperature and stirred for about one hour. Methanol was then removed and the residue was taken up in dichloromethane, washed with 1N hydrochloric acid solution, water and brine, and dried over magnesium sulfate. Purification by flash chromatography afforded lactone **6-12** as a white solid (0.25 g, 80%).

^{1}H NMR (500 MHz, CD_3COCD_3) δ 10.03 (br, 1H, NH of indole), 7.27-7.51 (m, 7H, phenyl protons, H-4 and H-7 of indole), 7.12 (m, 1H, H-6), 7.03 (m, 1H, H-5), 4.74-4.77 (dd, 1H, CHCOOC**H**$_2$CHN, J = 3.5 Hz and 10.5 Hz), 4.36 (d, 1H, CHCOOC**H**$_2$CHN, J = 10.5 Hz), 4.16 (m, 1H, CHCOOCH$_2$C**H**N), 4.09 (d, 1H, CH$_2$C**H**COOCH$_2$CHN, J = 5.5 Hz), 3.90 (d, 1H, C**H**$_2$Ph, J = 13.5 Hz), 3.78 (d, 1H, C**H**$_2$Ph, J = 13.5 Hz), 3.32-3.37 (dd, 1H, C**H**$_2$CHCOOCH$_2$CHN, J = 5.5 Hz and 16.5 Hz), 2.83 (d, 1H, C**H**$_2$CHCOOCH$_2$CHN, J = 16.5 Hz); ^{13}C NMR (125.7 MHz, CD_3COCD_3) δ 171.08, 138.78, 137.45, 130.60, 129.56, 129.27, 128.20, 127.63, 122.57, 119.99, 118.82, 112.19, 112.13, 107.71, 74.22, 59.10, 55.21, 49.82, 49.79, 22.61; MS (FAB) m/z 319 ((M+1)$^+$, 15.8%), 154 (100%).

Chiral auxiliary 6-29



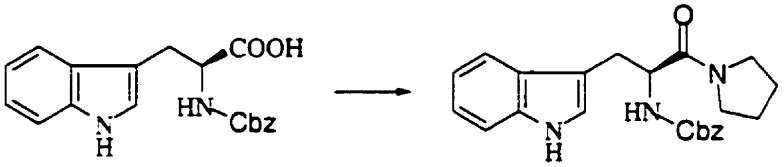
6-9 **6-29**

A mixture of N_b-benzyl-L-tryptophan methyl ester **6-9** (0.31 g, 1 mmol) and aldehyde **6-7** (0.23 g, 1.1 mmol) in toluene was brought to reflux with Dean-Stark water

trap to remove water. After 4 hours, the solvent was removed. The residue was dissolved in isopropylamine (20 ml) and heated at 80 °C overnight in a sealed vessel. The solvent was removed *in vacuo*, and the residue was taken up in dichloromethane, washed with water and brine, and dried over magnesium sulfate. Purification by flash chromatography using ethyl acetate/hexanes/triethyamine (2/8/1) as eluant provided the desired product **6-29** as light a yellow solid (0.24 g, 51%).

^{1}H NMR (500 MHz, CD_3COCD_3) δ 9.84 (br, 1H, NH of indole), 7.23-7.49 (m, 7H, phenyl protons, H-4 and H-7 of indole), 7.07 (m, 1H, H-6), 7.01 (m, 1H, H-5), 4.10 (m, 2H, CH_2**C**H**C**OOCH$_3$, NC**H**CH_2OH), 3.93 (d, 1H, NC**H**$_2$Ph, J = 14.5 Hz), 3.85 (m, 1H, CHC**H**$_2$OH), 3.80 (d, 1H, NC**H**$_2$Ph, J = 14.5 Hz), 3.70-3.73 (m, 4H, CHC**H**$_2$OH, COOC**H**$_3$), 3.10-3.15 (dd, 1H, C**H**$_2$CHCOOCH$_3$, J = 8.0 Hz and 15.5 Hz), 2.98-3.02 (dd, 1H, C**H**$_2$CHCOOCH$_3$, J = 5.0 Hz and 13.5 Hz), 2.90 (br, 1H, OH); ^{13}C NMR (125.7 MHz, CD_3COCD_3) δ 173.62, 140.77, 137.63, 133.92, 129.50, 129.09, 127.84, 121.84, 119.51, 118.59, 111.90, 107.39, 64.25, 58.71, 58.29, 54.63, 51.92, 22.33; MS (ES) m/z 349 (M-1).

N_b-Benzyloxycarbonyl-L-tryptophan pyrrolidine amide 6-16



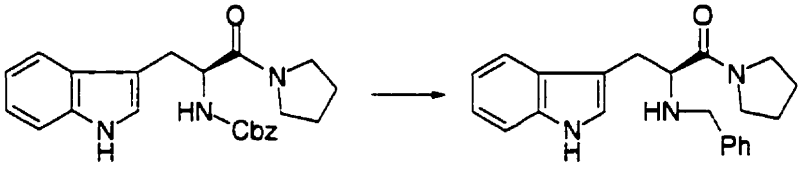
6-15 **6-16**

To a solution of N_b-benzyloxycarbonyl-L-tryptophan (1.02 g, 3 mmol), pyrrolidine (0.38 ml, 4.5 mmol) and triethylamine (0.46 ml, 3.3 mmol) in acetonitrile (15 ml) at room temperature was added *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium

hexafluorophosphate (HBTU) (1.25 g, 3.3 mmol). The resulting mixture was stirred overnight. The solvent was removed *in vacuo* and the residue was extracted with ethyl acetate (3x50 ml). The organic extracts were washed with brine, aqueous hydrochloric acid (2N) (5 ml), water (5 ml), saturated sodium bicarbonate (5 ml) and water (5 ml), and dried over magnesium sulfate. The solvent was removed to provide the desired product as a white solid (1.13 g, 96%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (br, 1H, NH of indole), 7.61 (d, 1H, H-4, J = 7.5 Hz), 7.34 (m, 6H, H-7 and aromatic protons of cbz), 7.16 (m, 1H, H-6), 7.11 (m, 1H, H-5), 7.04 (s, 1H, H-2), 5.80 (d, 1H, N**H**cbz), 5.10 (m, 2H, NCOOC**H**$_2$Ph), 4.72 (m, 1H, CH_2C**H**CON(C_4H_8)), 3.18-3.37 (m, 5H, C**H**$_2$CHCON(C_4H_8), 3H of pyrrolidine), 2.50 (m, 1H, 1H of pyrrolidine), 1.60 (m, 2H, 2H of pyrrolidine), 1.27-1.40 (m, 2H, 2H of pyrrolidine); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.02, 155.60, 136.29, 135.83, 129.51, 128.34, 128.27, 128.13, 127.90, 127.82, 127.35, 122.75, 121.96, 119.37, 118.61, 110.93, 110.51, 66.58, 53.43, 46.09, 45.72, 29.54, 25.43, 23.72; MS (FAB, NBA) m/z 392 ($(M+1)^+$, 59.3%), 243 (100%).

N_b-Benzyl-L-tryptophan pyrrolidine amide 6-18



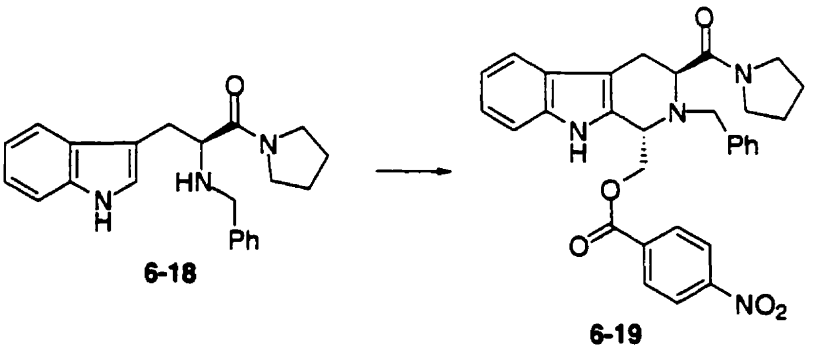
6-16 **6-18**

A hydrogenation flask was charged with N_b-benzyloxycarbonyl-L-tryptophan pyrrolidine amide **6-16** (1.13 g, 2.89 mmol) in a mixture of ethyl acetate (20 ml), water (1 ml) and acetic acid (3 drops). Palladium on carbon (10%) (0.5 g) was then added. The

solution was hydrogenated at 40 psi for 2 hours. The mixture was filtered through a glass sinter with celite. The crude product was taken up in chloroform and washed with saturated sodium bicarbonate, brine and dried over magnesium sulfate. The solvent was removed to give the crude product as a white solid in quantitative yield. To the crude amine in methanol (20 ml) was added benzaldehyde (0.32 ml, 3.18 mmol) and sodium cyanoborohydride (0.18 g, 2.89 mmol). The pH of the solution was adjusted to around 6 by adding acetic acid. The resulting mixture was then stirred overnight. Water was added to the reaction mixture until a white precipitate was formed. The pH of the solution was adjusted to around 8 with aqueous ammonia and it was extracted several times with chloroform. The combined organic extracts were washed with brine and dried over magnesium sulfate. The crude product was purified by flash chromatography to provide the desired **6-18** as a white solid (0.76 g, 76%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (m, 1H, NH of indole), 7.55 (d, 1H, H-4, J = 7.5 Hz), 7.21-7.24 (m, 6H, H-7, aromatic protons of benzyl), 7.15 (m, 1H, H-6), 7.07 (m, 1H, H-5), 7.01 (s, 1H, H-2), 3.84 (d, 1H, NCH_2Ph, J = 13.0 Hz), 3.61-3.65 (m, 2H, NCH_2Ph, $CH_2CHCON(C_4H_8)$), 3.38 (m, 1H, 1H of pyrrolidine), 3.25 (m, 1H, 1H of pyrrolidine), 3.14-3.18 (dd, 1H, $CH_2CHCON(C_4H_8)$, J = 5.5 Hz and 14.0 Hz), 3.03-3.08 (dd, 1H, $CH_2CHCON(C_4H_8)$, J = 9.5 Hz and 14.0 Hz), 2.86-2.91 (m, 1H, 1H of pyrrolidine), 2.37 (m, 1H, 1H of pyrrolidine), 2.29 (br, 1H, NHBn), 1.49-1.61 (m, 2H, 2H of pyrrolidine), 1.36-1.41 (m, 1H, 1H of pyrrolidine), 1.16-1.21 (m, 1H, 1H of pyrrolidine); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 173.27, 139.90, 135.99, 128.26, 128.24, 127.48, 126.91, 122.77, 121.83, 119.21, 118.75, 111.40, 111.89, 111.02, 60.11, 51.87, 45.75, 45.59, 29.91, 25.59, 23.83; MS (FAB, NBA) m/z 348 ($(M+1)^+$, 100%), 249 (44.3%), 217 (76.3%).

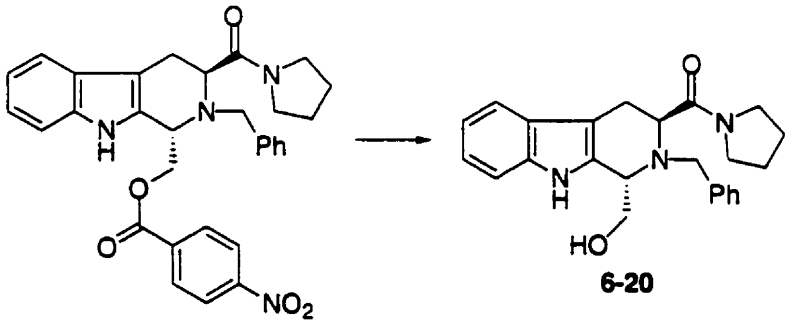
Compound 6-19



N_b-benzyl-L-tryptophan pyrrolidine amide **6-18** (0.35 g, 1 mmol) and 2-oxoethyl *p*-nitrobenzoate **6-6** (0.23 g, 1.1 mmol) were dissolved in toluene and the mixture was heated to reflux with Dean-Stark water trap to remove water. After 4 hours, the solution was concentrated to a small volume and then cooled down slowly to room temperature, providing a light yellow crystalline product **6-19** (0.32 g, 60%).

^{1}H NMR (500 MHz, CD_3COCD_3) δ 10.09 (br, 1H, NH of indole), 8.38 (d, 2H, protons of *para*-nitro benzene, J = 8.5 Hz), 8.07 (d, 2H, protons of *para*-nitro benzene, J = 8.5 Hz), 7.56 (d, 1H, H-4, J = 8.0 Hz), 7.37 (d, 1H, H-7, J = 8.0 Hz), 7.18-7.26 (m, 5H, aromatic protons of benzyl), 7.12 (m, 1H, H-6), 7.06 (m, 1H, H-5), 4.66 (m, 2H, BnNCHC**H**$_2$OCO), 4.44 (m, 1H, 1H of pyrrolidine), 4.32 (m, 2H, BnNC**H**CH$_2$OCO, CH$_2$C**H**CON(C_4H_8)), 3.92 (d, 1H, NC**H**Ph, J = 14.0 Hz), 3.53-3.60 (m, 5H, NC**H**Ph, C**H**$_2$CHCON(C_4H_8), 3H of pyrrolidine), 2.63-2.67 (dd, 1H, C**H**$_2$CHCON(C_4H_8), J = 4.5 Hz and 16.5 Hz), 1.85 (m, 4H, 4H of pyrrolidine); ^{13}C NMR (125.7 MHz, CD_3COCD_3) δ 170.00, 164.83, 151.65, 140.70, 137.87, 137.72, 136.14, 131.52, 129.85, 129.07, 127.73, 124.46, 122.37, 119.71, 118.95, 111.99, 111.95, 65.99, 56.79, 56.17, 53.00, 46.72, 46.38, 26.86, 24.87, 19.31; MS (FAB, NBA) m/z 539 ((M+1)$^+$, 7.6%). 154 (100%).

Chiral auxiliary 6-20

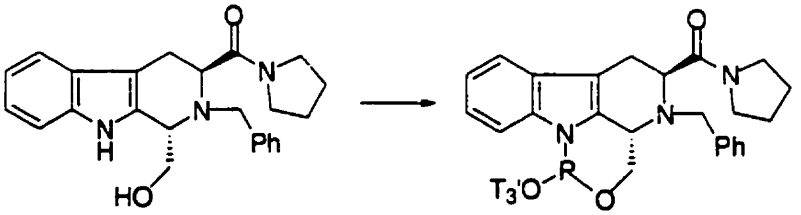


6-19 **6-20**

A solution of **6-19** (110 mg, 0.2 mmol) in 2N aqueous sodium hydroxide (3 ml) and tetrahydrofuran (3 ml) was warmed up to 55 °C for one hour. Tetrahydrofuran was then evaporated and the residue was extracted several times with ethyl acetate. The combined organic extracts were washed with water, brine and dried over magnesium sulfate. The solvent was removed to afford the desired product **6-20** as a white solid (74 mg, 95%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (br, 1H, NH of indole), 7.56 (d, 1H, H-4, 7.5 Hz), 7.10-7.30 (m, 8H, H-5, H-6, H-7 of indole and aromatic protons of benzyl), 4.10 (m, 2H, $NC\mathbf{H}_2Ph$), 4.01-4.03 (m, 2H, $CH_2C\mathbf{H}CON(C_4H_8)$, 1H of pyrrolidine), 3.90-3.96 (m, 1H, $BnNCH_2C\mathbf{H}OH$), 3.72-3.80 (m, 2H, $BnNC\mathbf{H}_2CHOH$), 3.40-3.58 (m, 4H, $C\mathbf{H}_2CHCON(C_4H_8)$, 3H of pyrrolidine), 2.72-2.76 (dd, 1H, $C\mathbf{H}_2CHCON(C_4H_8)$, J = 4.0 Hz and 16.5 Hz), 1.87-2.03 (m, 4H, 4H of pyrrolidine); ^{13}C NMR (125.7 MHz, CD_3COCD_3) δ 169.79, 139.32, 136.17, 130.35, 128.58, 128.42, 127.12, 126.83, 121.83, 119.40, 118.27, 110.73, 108.66, 63.71, 57.75, 56.86, 52.75, 46.17, 26.30, 23.99, 19.04, 14.02; MS (FAB, NBA) m/z 390 ($(M+1)^+$, 43.1%), 371 (22.5%), 154 (100%).

Cyclic Indole-oxazaphosphorine 6-22



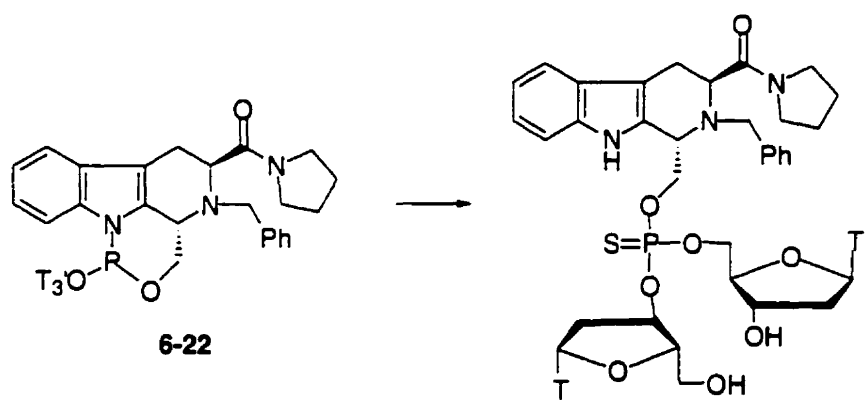
6-20 **6-22**

To a dichloromethane (0.25 ml) solution of phosphorus trichloride (8.7 µl, 0.1 mmol) in a scrupulously dried NMR tube at 0 °C was added slowly a solution of chiral auxiliary **6-20** (39 mg, 0.1 mmol) in dry dichloromethane containing triethylamine (30.7 µl, 0.22 mmol). The NMR tube was then sealed. Initially ^{31}P NMR indicated several resonances. After heating at 50 °C for 24 hours, a major resonance at around 144 ppm was observed. A solution of 5'-O-TBDMS-thymidine (39 mg, 0.11 mmol) and triethylamine (15.4 µl, 0.11 mmol) was added to the NMR tube at 0 °C, the NMR tube was re-sealed and warmed up slowly to room temperature. When ^{31}P NMR showed the disappearance of resonance at 144 ppm and the formation of new resonance at around 118 ppm, the solvent was removed *in vacuo*. The residue was purified by flash chromatography to afford the desire cyclic indolo-oxazaphosphorine **6-22** as a white solid (39 mg, 51%).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 116.96 ppm; ^1H NMR (500 MHz, $CDCl_3$) δ 8.48 (br, 1H, NH), 7.54 (d, 1H, H-4, J_{4-5} = 7.5 Hz), 7.51 (d, 1H, H-7, J_{6-7} = 7.5 Hz), 7.45 (s, 1H, C**H**=C(CH_3)), 7.19-7.35 (m, 7H, phenyl protons, H-5 and H-6), 6.12 (m, 1H, H-1'), 4.90 (m, 1H, H-3'), 4.36 (m, 1H, OC**H**$_2$CHNBn), 3.45-4.00 (m, 8H, NC**H**$_2$Ph, CH_2C**H**CONC$_4$H$_8$, H-4', H-5', OC**H**$_2$C**H**NBn), 3.50 (m, 3H, 3H of pyrrolidine), 3.26 (m, 1H, 1H of pyrrolidine), 3.13 (m, 1H, C**H**$_2$CHCONC$_4$H$_8$), 2.96 (m, 1H,

$CH_2CHCONC_4H_8$), 2.23 (m, 1H, H-2'), 2.01-2.06 (m, 1H, H-2'), 1.82-1.95 (m, 7H, 4H of pyrrolidine), (CH_3)C=C), 0.91 (s, 9H, Si(C(CH_3)$_3$), 0.08 (2s's, 6H, Si(CH_3)$_2$(*t*-Bu)); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.39, 163.49, 149.81, 138.70, 135.25, 132.75, 128.45, 127.31, 122.39, 121.20, 118.53, 111.01, 110.55, 109.23, 85.72 (d, J_{C-P} = 3.77 Hz), 84.42, 72.61 (d, J_{C-P} = 10.2 Hz), 67.75 (d, J_{C-P} = 9.2 Hz), 62.12, 60.24, 56.79, 52.45 (d, J_{C-P} = 8.2 Hz), 45.97, 45.68, 39.40, 26.17, 25.78, 23.95, 21.26, 18.20, 14.03, 12.42, -5.67, -5.64; MS (FAB, NBA) m/z 774 ((M+1)$^+$, 15%).

Dithymidyl Indo-phosphorothioate 6-25

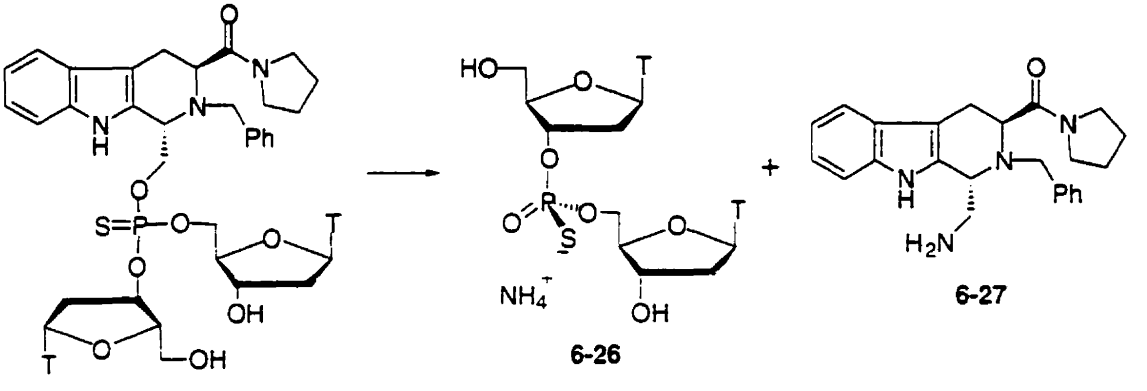


To a stirred solution of cyclic indolo-oxazaphosphorine **6-22** (38.6 mg, 0.05 mmol) and 3'-O-TBDMS-thymidine (35.6 mg, 0.1 mmol) in dry THF (2 ml) at room temperature was added DBU (0.25 mmol, 37 μl). After 30 minutes, ^{31}P NMR of the reaction mixture showed a single resonance at around 140 ppm. The mixture was then loaded to a short column to filter off DBU. After evaporation of the solvent, the residue was dissolved in dry dichloromethane and Beaucage's sulfurizing reagent (10 mg, 0.05 mmol) was added at room temperature. After stirring for 5 minutes, ^{31}P NMR revealed a single resonance at around 68 ppm. The solvent was removed, and the residue was

dissolved in THF, and tetrabutylammonium fluoride (TBAF) was then added. After 2 hours, the solvent was removed *in vacuo*. Dichloromethane was added to the residue, washed with water and brine, and dried over magnesium sulfate. Purification by column chromatography afforded **6-25** as a white solid (28 mg, 61%).

^{31}P NMR (202.3 MHz, CD_3COCD_3) δ 66.87, 66.98 ppm (20:1); ^{1}H NMR (500 MHz, CD_3COCD_3) δ 10.07 (br, 1H), 10.02 (br, 1H), 9.94 (br, 1H), 7.71 (s, 1H), 7.53 (d, 1H, J = 8.0 Hz), 7.47 (s, 1H), 7.18-7.34 (m, 6H), 7.08 (t, 1H), 7.02(t, 1H), 6.26-6.33 (m, 2H), 5.17 (m, 1H), 4.02-4.52 (m, 11H), 3.93 (d, 1H, J =14.5 Hz), 3.64-3.73 (m, 4H), 3.47-3.51 (m, 2H), 3.33-3.39 (m, 3H), 2.62 (m, 1H), 2.22-2.38 (m, 4H), 1.77-1.87 (m, 8H); ^{13}C NMR (125.7 MHz, CD_3COCD_3) δ 169.96, 164.16, 164.10, 151.39, 151.26, 140.51, 137.71, 136.51, 129.75, 129.55, 129.10, 128.05, 127.71, 122.31, 119.67, 118.90, 111.69, 111.20, 111.14, 110.15, 86.47, 86.42, 85.63, 85.35, 81.10, 81.06, 71.84, 69.04, 68.99, 68.96, 68.91, 62.61, 60.49, 57.40, 57.31, 57.17, 53.18, 47.01, 46.80, 40.16, 38.92, 38.89, 27.00, 24.85, 19.26, 14.44, 12.61, 12.58; MS (FAB, NBA) m/z 934 $(M+1)^+$.

Synthesis of *Rp*-ammonium thymid-3'-yl thymid-5'-yl phosphorothioate 6-26 and amino chiral auxiliary 6-27



6-25 **6-26** **6-27**

Phosphorothioate **6-25** (28 mg, 0.026 mmol) from previous step was dissolved in concentrated ammonia/ethanol (3/1) (5 ml). The mixture was heated at 55 °C for 16 hours. ^{31}P NMR shifted quantitatively from 68 ppm to 56 ppm. The reaction mixture was extracted with chloroform several times. The combined organic extracts were washed with brine and dried over magnesium sulfate. The solvent was removed to provide **6-27** (9.2 mg, 91%) as a light yellow solid. The aqueous crude was purified by flash chromatography to give **6-26** (15 mg, 89%).

6-26: ^{31}P NMR (202.3 MHz, CD_3OD) δ 56.10, 56.02 ppm (1:20); ^{1}H NMR (500 MHz, CD_3OD) δ 7.90 (s, 1H, ^{5}H-6), 7.85 (s, 1H, ^{3}H-6), 6.35 (dd, 1H, ^{5}H-1', J = 8.0 Hz, 6.5 Hz), 6.28 (dd, 1H, ^{3}H-1', J = 7.8 Hz, 6.0 Hz), 5.05 (m, 1H, ^{3}H-3'), 4.51 (m, 1H, ^{5}H-3'), 4.20 (m, 1H, ^{3}H-4'), 4.13 (m, 2H, ^{5}H-5'), 4.03 (m, 1H, ^{5}H-4'), 3.83 (m, 2H, ^{3}H-5'), 2.44 (m, 1H, ^{5}H-2'), 2.25 (m, 2H, ^{3}H-2'), 2.19 (m, 1H, ^{5}H-2'), 1.97 (s, 3H, CH=C(C**H**$_3$)), 1.88 (s, 3H, CH=C(C**H**$_3$)); ^{13}C NMR (125.7 MHz, CD_3OD) δ 166.45, 166.31, 152.43, 152.26, 138.17, 138.11, 112.07, 111.49, 87.90 (d, J = 5.5 Hz), 87.49 (d, J = 8.8 Hz), 86.25, 86.14, 77.62 (d, J = 5.0 Hz), 72.97, 66.33 (d, J = 6.3 Hz), 62.91, 40.97, 39.90 (d, J = 5.0 Hz), 12.64, 12.42; MS (FAB-, NBA) m/z 561 (dTP(S)(O)dT)$^-$).

6-27: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, 1H, J = 8.0 Hz), 7.23-7.35 (m, 6H), 7.13 (m, 1H), 7.02 (m, 1H), 4.71 (br, 1H), 4.04-4.14 (m, 2H), 3.94 (m, 2H), 3.68-3.86 (m, 2H), 3.11-3.23 (m, 4H), 2.97 (m, 1H), 2.82 (m, 1H), 1.55-1.82 (m, 5H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 173.49, 138.89, 136.28, 129.02, 128.28, 127.36, 127.30, 122.05, 118.89, 117.24, 112.02, 108.93, 62.97, 61.89, 53.74, 52.41, 48.68, 45.93, 29.26, 26.12, 23.65, 22.29; LRMS (FAB, NBA) m/z 411 $(M+Na)^+$, 389 $((M+1)^+$, 67.2%), 372 (100%); HRMS (FAB) $C_{24}H_{29}N_4O$ $((M+1)^+)$ calc: 389.2341, found: 389.2343.

The loading of thymidine on CPG (6-37)

6-36 6-37

A mixture of 5'-O-DMTr-thymidine (55 mg, 0.1 mmol), CPG with sarcosinyl-succinonyl linker (0.5 g), 4-dimethylaminopyridine (DMAP) (6 mg, 0.05 mmol), triethylamine (40 μl), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DEC) (0.19 g, 1mmol) and anhydrous pyridine (6 ml) in a hypovial was shaken at room temperature for 24 hours. Pentachlorophenol (67 mg, 0.25 mmol) was then added, and the mixture was shaken for an additional period of 16 hours. The CPG was filtered off and washed successively with pyridine, dichlormethane and ether. Reagent grade piperidine (2 ml) was added, the resulting slurry was shaken for 5 minutes. The CPG was again filtered off and washed with dichloromethane and ether, respectively, and dried under vacuum. The dry CPG was mixed with equal parts of two solutions of 0.5 M acetic anhydride in THF and 0.5 M DMAP/2,4,6-trimethylpyridine in THF (2 ml each). The slurry was shaken for 2 hours, then washed successively with pyridine, dichloromethane, THF and ether. Drying under vacuum afforded the immobilized thymidine (0.49 g). Portions of this CPG were re-capped on the DNA synthesizer prior to the synthesis.

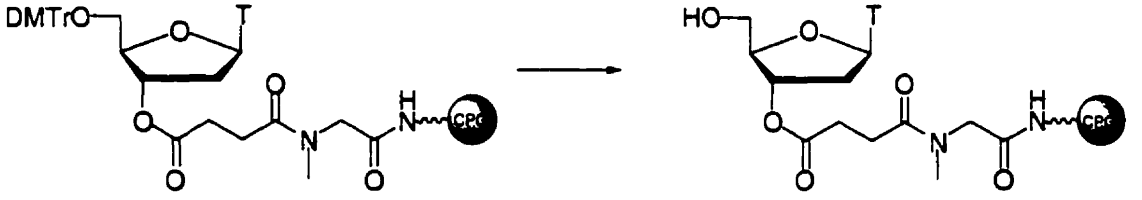
The loading amount was measured by trityl analysis. Three portions of CGP around 5 mg were weighed out precisely into three vials, trichloroacetic acid (TCA) (3% in dichloroethane) (5ml) was added into each vial. The absorbances of the solutions at

504 nm were measured by CARY 300 Bio UV-Visible Spectrophotometer, loading amount was calculated according to the following equation:

L = Abs x Vol of TCA (ml) x 1000/ mg of CPG x ε, where ε equals to 76 ml/cm•μmol

And accordingly the loading amount determined by this method was 31.03 μmol/g.

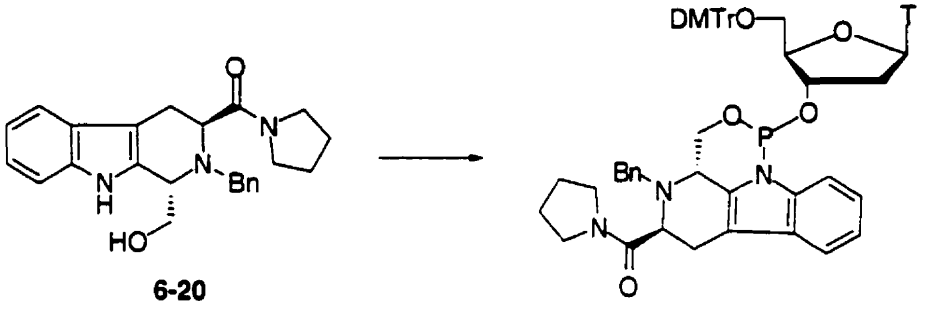
The immobilized thymidine 6-40



6-37 **6-40**

The immobilized thymidine **6-37** (32.3 mg, 1 μmol) was placed in a sintered glass funnel and washed with trichloroacetic acid solution until no orange color was observed, CPG was further washed with dry acetonitrile several times. The resulting CPG was then dried under vacuum to afford 5'-hydroxy immobilized thymidine **6-40**.

Indolo-oxazaphosphorine 6-38



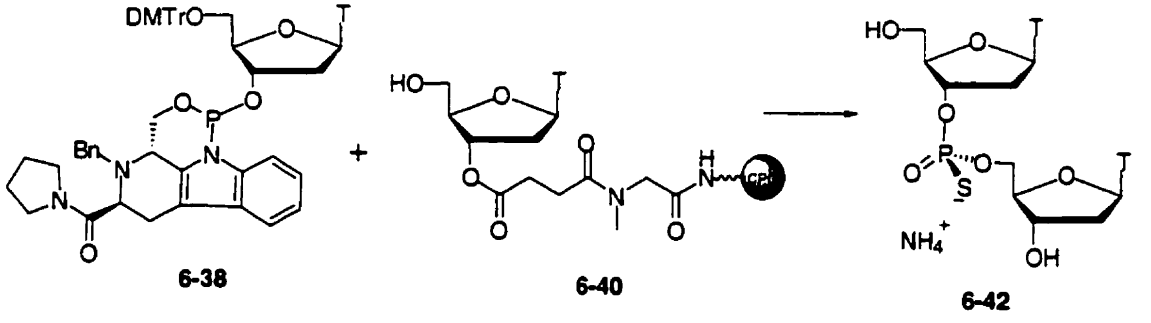
6-20 **6-38**

To a dichloromethane (0.25 ml) solution of phosphorus trichloride (8.7 μl, 0.1 mmol) in a scrupulously dried NMR tube at 0 °C was added slowly to a solution of chiral

auxiliary (39 mg, 0.1 mmol) in dry dichloromethane containing triethylamine (30.7 µl, 0.22 mmol). The NMR tube was then sealed. Initially ^{31}P NMR indicated several resonances. After heating at 50 °C for 24 hours, a major resonance at around 144 ppm was observed. A solution of 5'-O-DMTr-thymidine (60 mg, 0.11 mmol) and triethylamine (15.4 µl, 0.11 mmol) was added to the NMR tube at 0 °C, the NMR tube was re-sealed and warmed slowly to room temperature. After 20 minutes, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography to afford the desired product as a white solid (43 mg, 45%).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 118 ppm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11-7.56 (m, 18H), 6.83 (d, 4H, J = 8.5 Hz), 6.18 (m, 1H), 5.12 (m, 1H), 4.89 (m, 1H), 4.27 (m, 1H), 3.93-4.06 (m, 5H), 3.11-3.85 (m, 8H), 2.95 (m, 2H), 2.34 (m, 2H), 1.82-1.96 (m, 8H), 1.49 (m, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.54, 163.61, 158.64, 158.61, 149.97, 144.34, 138.83, 138.72, 138.59, 135.49, 135.34, 135.29, 132.93, 130.08, 130.03, 128.58, 128.55, 128.07, 127.99, 127.46, 127.05, 122.55, 121.35, 118.61, 113.26, 111.26, 111.18, 110.99, 110.87, 109.44, 86.79, 84.66, 84.63, 84.40, 72.65, 67.79, 67.73, 63.81, 62.12, 60.37, 56.88, 55.19, 53.10, 52.84, 52.70, 52.63, 46.28, 46.09, 45.83, 39.40, 26.29, 24.08, 21.37, 21.04, 19.12, 14.16, 11.87, 11.43; MS (FAB, NBA) m/z 984 $(M+Na)^+$.

Ammonium *Rp*-thymid-3'-yl thymid-5'-yl phosphorothioate 6-42



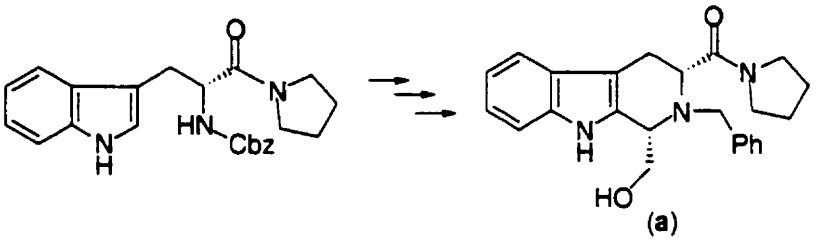
6-38 6-40 6-42

5'-Hydroxy immobilized thymidine (1 μmmol) on CPG in the sintered glass funnel was dried under vacuum overnight. DBU (15 μl, 0.1 mmol) in dry acetonitrile (0.5 ml) was syringed to the sintered glass funnel under argon at room temperature. After 1 minute, the indole-oxazaphosphorine monomer (19 mg, 0.02 mmol) in dry acetonitrile (0.5 ml) was added slowly. The resulting mixture was allowed to stand at room temperature for 20 minutes. The solid support was then washed with acetonitrile (3 x 2 ml) and treated with Beaucage's sulfurizing reagent (4 mg, 0.02 mmol) in dry acetonitrile (0.5 ml). After 10 minutes, the solid support was washed with acetonitrile (3 x 2 ml). After detritylation with trichloroacetic acid (TCA) in 1,2-dichloroethane till no orange color was observed, the CPG was transferred into a small flask and treated with concentrated ammonia/ethanol (v/v=3/1) at 55 °C for 16 hours. The CPG was filtered off, the filtrate was evaporated to dryness, distilled water was added to the residue. The product was confirmed by HPLC, by comparison with a mixture of *Rp* and *Sp* diastereomers obtained by non-stereoselective method (retention time 14.62 min and 16.86 min for *Rp* and *Sp* isomers, respectively).

HPLC: Retention time 14.62 min; (Phenomenex C8 Column; solvent A: water; solvent B: acetonitrile; flow rate: 1.5 ml/min; 3% B increases linearly to 7% for the first 30

minutes, then increases to 40% during the next 20 minutes); HPLC-MS (–ev) m/z 561 ((dTP(S)(O)dT)$^-$).

Chiral auxiliary derived from D-tryptophan (a)

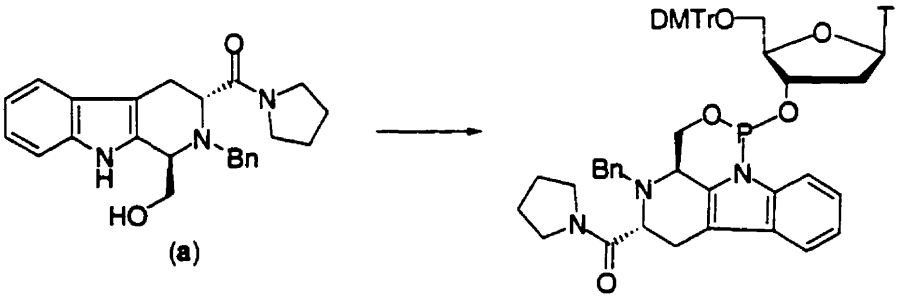


(a)

D-Tryptophan derived chiral auxiliary (**a**) was prepared using the same procedure as described for the preparation of **6-20**.

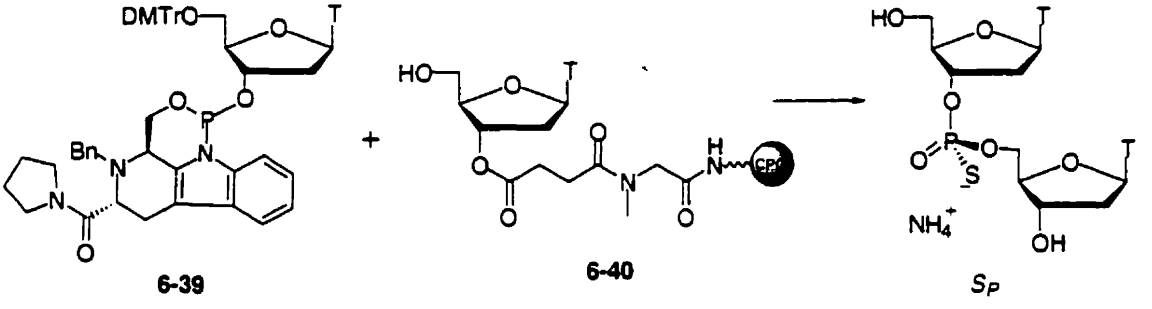
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.74 (br, 1H), 7.51 (d, 1H, J = 7.5 Hz), 7.20-7.34 (m, 6H), 7.06 (t, 1H), 7.01 (t, 1H), 4.30-4.35 (m, 1H), 4.25-4.28 (dd, 1H, J = 4.5 Hz and 11.0 Hz), 3.91-3.95 (m, 2H), 3.80-3.86 (m, 2H), 3.61-3.71 (m, 2H), 3.31-3.50 (m, 3H), 2.55-2.59 (dd, 1H, J = 4.5 Hz and 16.5 Hz), 1.84-2.07 (m, 4H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.34, 141.15, 137.66, 132.78, 129.42, 129.06, 128.20, 127.56, 121.74, 119.37, 118.63, 111.80, 108.60, 64.84, 64.74, 60.49, 59.44, 53.30, 46.71, 46.66, 27.01, 24.83, 20.78, 19.53, 14.45; MS (FAB, NBA) m/z 390 ((M+1), 45.2%), 154 (100%).

Indolo-oxazaphosphorine 6-39



Indolo-oxazaphosphorine **6-39** was prepared using the same procedure as described for the preparation of **6-38**. The crude product was used for the solid phase synthesis without further purification.

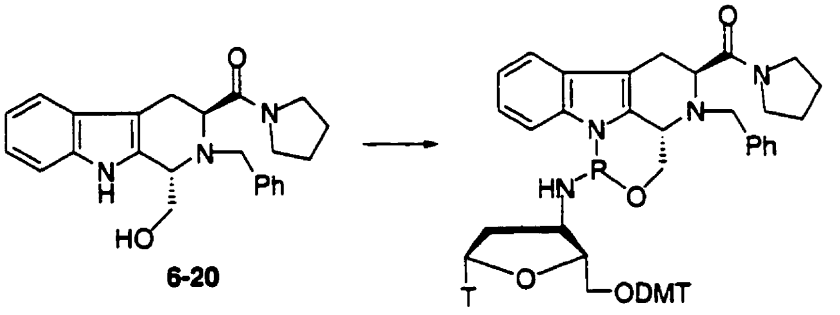
Ammonium *Sp*-thymid-3'-yl thymid-5'-yl phosphorothioate



S_P- Dithymidine phosphorothioate was prepared using the same protocol as described for the preparation of **6-42**.

HPLC: Retention time 16.86 min; (Phenomenex C8 Column; solvent A: water; solvent B: acetonitrile; flow rate: 1.5 ml/min; 3% B increase linearly to 7% for the first 30 minutes, then increase to 40% during the next 20 minutes); HPLC-MS (–ev) m/z 561 ((dTP(S)(O)dT)$^-$).

N3'-P5' phosphoramidite 6-45



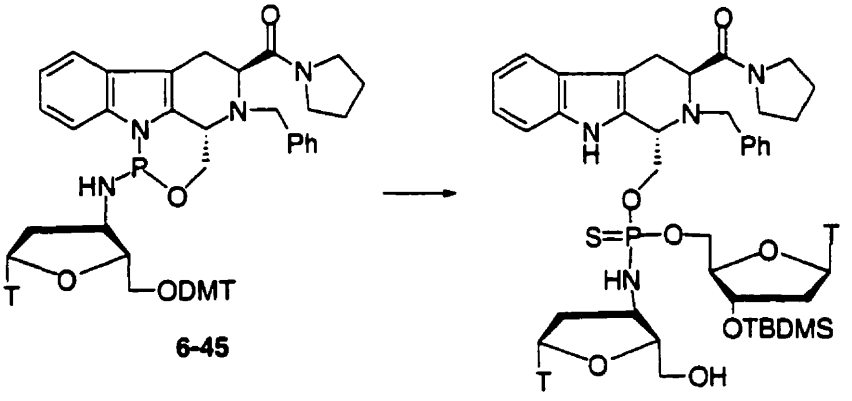
6-20 **6-45**

To a dichloromethane (0.25 ml) solution of phosphorus trichloride (8.7 µl, 0.1 mmol) in a scrupulously dried NMR tube at 0 °C was added slowly a solution of chiral auxiliary **6-20** (39 mg, 0.1 mmol) in dry dichloromethane containing triethylamine (30.7 µl, 0.22 mmol). The NMR tube was then sealed. Initially ^{31}P NMR indicated several resonances. After heating at 50 °C for 24 hours, a major resonance at around 144 ppm was observed. A solution of 5'-O-DMTr-3'-NH_2-thymidine (59.8 mg, 0.11 mmol) and triethylamine (15.4 µl, 0.11 mmol) was added to the NMR tube at 0 °C. The NMR tube was re-sealed and warmed slowly to room temperature. When ^{31}P NMR showed the disappearance of resonance at 144 ppm and the formation of new resonance at around 118 ppm, the solvent was removed *in vacuo*. The residue was purified by flash chromatography to afford the desire cyclic **6-45** as a white solid (40 mg, 42%).

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 119.0 ppm; ^{1}H NMR (500 MHz, $CDCl_3$) 7.13-7.52 (m, 18H), 6.87 (d, 4H, J = 7.5 Hz), 6.25 (m, 1H,), 5.07 (m, 1H), 4.32-4.40 (m, 2H), 3.66-3.77 (m, 6H), 2.90-3.56 (m, 6H), 1.76-1.93 (m, 4H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 171.90, 164.17, 159.12, 150.87, 145.21, 139.65, 136.20, 136.12, 135.62, 130.50, 128.91, 128.75, 128.59, 128.29, 127.70, 127.23, 122.18, 121.08, 118.40, 113.54, 112.09, 112.02, 111.30,

108.38, 86.86, 85.63, 85.60, 84.07, 67.99, 62.46, 60.58, 56.23, 55.72, 55.56, 55.52, 50.32, 50.25, 46.48, 46.42, 41.06, 26.60, 24.41, 22.97, 21.14, 14.33, 12.20, 11.81.

Thiophosphoramidate 6-47



To a stirred mixture of N3'-P5' phosphoramidite **6-45** (48 mg, 0.05 mmol) and 3'-O-TBDMS-thymidine (35.6 mg, 0.1 mmol) in dry THF (2 ml) at room temperature was added DBU (0.25 mmol, 37 μl). The mixture was heated at 55 °C for 2 hours. The mixture was then loaded to a short column to filter off DBU. After evaporation of the solvent, the residue was dissolved in dry dichloromethane and Beaucage's sulfurizing reagent (10 mg, 0.05 mmol) was added at room temperature. After stirring for 5 minutes, the mixture was then treated with 80% aqueous acetic acid for half an hour. The pH of the solution was adjusted to around 7 by adding sodium carbonate, extracted with ethyl acetate. The combined organic extracts were washed with water and brine, and dried over magnesium sulfate. Purification by flash chromatography afforded **6-47** as a white solid (36.6 mg, 35%).

^{31}P NMR (202.3 MHz, CD_3COCD_3) δ 73.28 ppm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (br, 1H), 7.54 (d, 1H, J = 7.5 Hz), 7.21-7.31 (m, 8H), 7.05-7.10 (m, 2H), 6.07 (m, 1H),

5.88 (m, 1H), 4.21-4.34 (m, 4H), 4.08 (m, 6H), 3.27-3.59 (m, 10H), 2.73-2.77 (m, 1H), 2.47-2.56 (m, 2H), 1.99-2.01 (m, 1H), 1.84-1.89 (m, 6H), 1.10-1.19 (m, 4H), 0.88 (s, 9H), 0.084 (s, 3H), 0.079 (s, 3H); ^{13}C NMR (125.7 MHz, CD_3COCD_3) δ 169.57, 163.98, 163.33, 150.56, 149.97, 139.36, 137.84, 136.18, 135.18, 129.58, 128.45, 128.28, 126.97, 126.73, 121.71, 119.21, 118.30, 111.32, 110.84, 109.00, 88.99, 85.73, 85.39, 85.34, 84.11, 71.66, 68.06, 66.39, 62.00, 57.11, 55.51, 52.71, 52.28, 47.37, 46.26, 46.19, 45.93, 42.67, 39.79, 39.04, 38.26, 27.63, 26.27, 25.53, 23.99, 21.29, 18.85, 17.73, 14.70, 12.44, 12.25; MS (FAB, NBA) m/z 1047 ($(M+1)^+$, 11.2%), 102.7 (100%).