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The Stereoselective Synthesis of Phosphorothioates and Methylphosphonates

by

Jian-Chao Wang

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

Doctor of Philosophy

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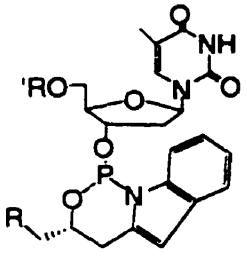
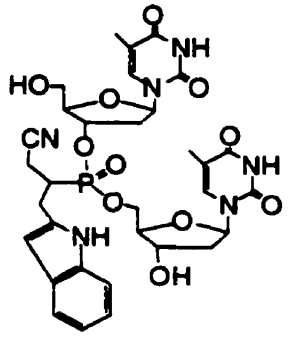
Abstract

The diastereoselective synthesis of phosphorothioates and methylphosphonates has been investigated. It has been found that indole is a good leaving group, and that it can form a stable indolephosphorine and can be stereospecifically substituted by a nucleoside in the presence of DBU.

Chiral indole-oxazaphosphorines **31**, **39**, **44**, **56** were synthesized and their reactivities were investigated. The cyano derivative **56** turned out to be a good chiral precursor in the diastereoselective synthesis of a T-T phosphorothioate dimer **65**, with a diastereomeric excess larger than 96%. The reaction of cyano monomer **63** on solid support was investigated. It was found that alkylphosphonate **64** was obtained as the major product, rather than the expected phosphorothioate **65**.



31 R' = TBDMS, R = H
39 R' = TBDMS, R = TBDMSO
44 R' = TBDMS, R = $NAcCH(CH_3)_2$
56 R' = TBDMS, R = CN
63 R' = DMTr, R = CN



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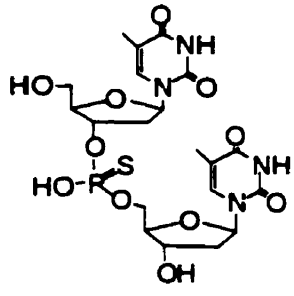
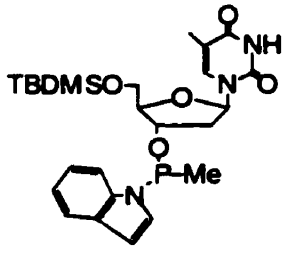


65

A novel internucleoside coupling reagent **87** was developed for the synthesis of methylphosphonates, in which the indole group can be replaced by a nucleoside within several minutes in the presence of DBU. Several chiral auxiliaries were tested for the stereoselective synthesis of methylphosphonates. A diastereomerically enriched monoester **104** (66% de) was synthesized.



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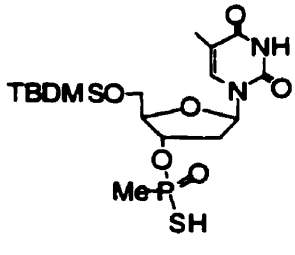

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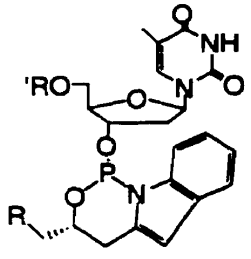
Résumé

La synthèse diastéréosélective de phosphorothioates et méthylphosphonates a été étudiée. Nous avons montré que l'indole est un bon groupement partant capable de former une indole-phosphorine stable pouvant être substituée de façon stéréospécifique par un nucléoside en présence de DBU.

Les indoles oxazaphosphorines chirales **31**, **39**, **44** et **56** ont été synthétisées et leurs réactivités étudiées. Le dérivé **56** porteur d'un groupement CN s'est révélé un bon précurseur pour la synthèse diastéréosélective d'un dimère phosphorothioate T-T **65** avec un excès diastéréoisomérique supérieur à 96%. La réaction avec le monomère **63** a été étudiée sur support solide. Nous avons montré que l'alkylphosphonate **64** est obtenu majoritairement au lieu du phosphorothioate attendu **65**.



31 R' = TBDMS, R = H
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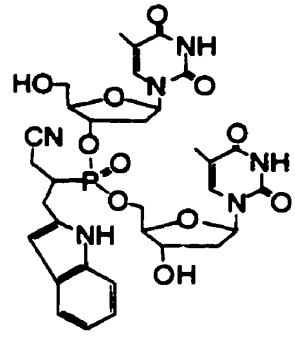

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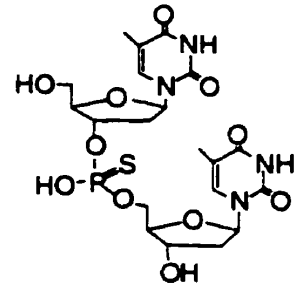
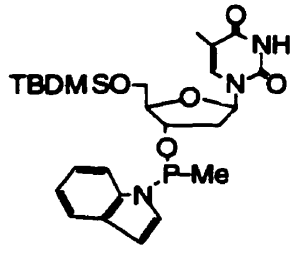


65

Un nouveau réactif de couplage internucléosidique **87** a été développé pour la synthèse de méthylphosphonates dans lequel le groupe indole peut être remplacé par un nucléoside en quelques minutes en présence de DBU. Plusieurs auxiliaires chiraux ont été testés pour la synthèse stéréosélective de méthylphosphonates. Le monoester **104** diastéréoisomériquement enrichi (66% ed) a été synthétisé.



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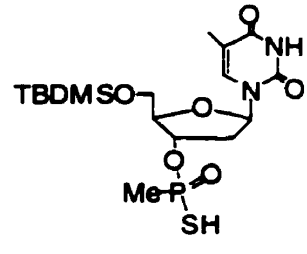

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I thank all my past and present co-workers in Dr. Just's group for their cooperation and helpful discussions. Special thanks to Mr. Toni Tomasino, Dr. Marija Prhavc, Dr. Nazim Mohamed and Mr. Ulhas Bhatt for the help in correcting my English, and Mr. Lu Jiang, Dr. Xiaoming Zhang, and Dr. Jiahua Chen for help in HPLC and UV measurements.

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Glossary of Abbreviations

A	adenine
Ac	acetyl
AIDS	acquired immunodeficiency syndrome
Ar	aryl
b	broad (NMR)
B	base
BDT	1,3-benzodithiol-2-yl
b.p.	boiling point
Bu	*n*-butyl
c	concentration (for the measurement of optical rotation)
C	cytosine
C	Celsius
calcd	calculated
CI	chemical ionization
COSY	correlation spectroscopy
CPG	controlled pore glass
δ	chemical shift
d	doublet (in NMR)
dA	2'-deoxyadenosine
DBU	1,8-diazabicyclo[5,4,0]undec-1-ene
DCC	dicyclohexylcarbodiimide
de	diastereomeric excess
DEC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
DECP	diethyl phosphorochloridate
DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide

MMTr	monomethoxytrityl [(4-methoxyphenyl)diphenylmethyl]
m.p.	melting point
mRNA	*messenger* ribonucleic acid
Ms	mesyl (methanesulfonyl)
MS	mass spectrometry
N	normal (solution)
NBA	nitrobenzyl alcohol
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
OMP	oligodeoxyribonucleoside methylphosphonates
OPS	oligoncleoside phosphorothioates
p-	para
PKC	protein kinase C
Ph	phenyl
PNAs	peptide nucleic acids
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(s)
t	triplet (NMR)
T	thymine
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
tBu	*tert*-butyl
TFA	trifluoroacetic acid

THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
$T^{3'}OH$	5'-O-TBDPS-thymidine
$T^{5'}OH$	3'-O-TBDMS-thymidine
TPSCl	2,4,6-triisopropylbenzenesulfonyl chloride
Tr	trityl (triphenylmethyl)
tRNA	*transfer* ribonucleic acid
Ts	tosyl (*para*-toluenesulfonyl)
µl	microliter
µmol	micromole
UV	ultraviolet
v	volume
w	weight

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Chapter I. Introduction and Literature Survey

1.1. Antisense Strategy

For most of the 20th century, hopes to find new medicines focused on active molecules interacting on the level of proteins. In many cases, 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 and often incompletely understood. On average, it is necessary to synthesize and test about 10,000 new compounds in order to discover a new active substance worth development. Recently, as our understanding of genetic science is increasing, we slowly gain insight on the genetic level of diseases. Attempts are being made to design drugs that will bind to selected sites on the nucleic acids (DNA and RNA) that direct the synthesis of disease related proteins.

As we know, for a protein to be made, the unique gene that specifies its composition must be expressed. That is the gene must expressed, or copied, from double-strand DNA into individual molecules of single-strand messenger RNA. Then the messenger RNA molecules must be translated into the specified protein.[1] The flow of genetic information in normal cells is

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{RNA} \xrightarrow{\text{Translation}} \text{Protein}$$

If a compound can combine with chosen segments of messenger RNA, it will impede translation of selected genes. In so doing, it will prevent deleterious proteins from being made at all, as shown in Figure 1.[2,3] The specific binding of a nucleic acid or nucleic acid analogue to a mRNA to prevent its expression has been termed antisense strategy.

---

[1] Lubert Stryer, Biochemistry, 4th Ed., W. H. Freeman and Company New York, **1995**, pp 95.
[2] Weintraub, H. M. *Scientific American* **1990**, *1*, 40.
[3] Cohen, J. S.; Hogan, M. E., *Scientific American* **1994**, *12*, 76.

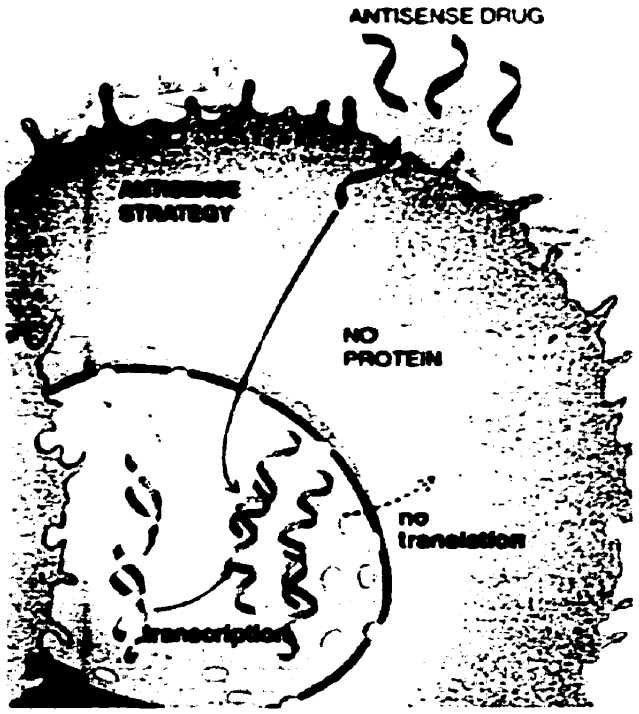


Figure 1. Antisense Strategy

The nucleic acid binding agents receiving the most study are DNA oligonucleotides, which interact with complementary nucleic acids forming a double helix. The complementarity is achieved by hydrogen bonds between Watson-Crick bases.[4] Adenine (A) is always paired with thymine (T), and guanine (G) is always paired with cytosine (C) (Figure 2). The oligonucleotides having antisense activities are called antisense oligonucleotides.

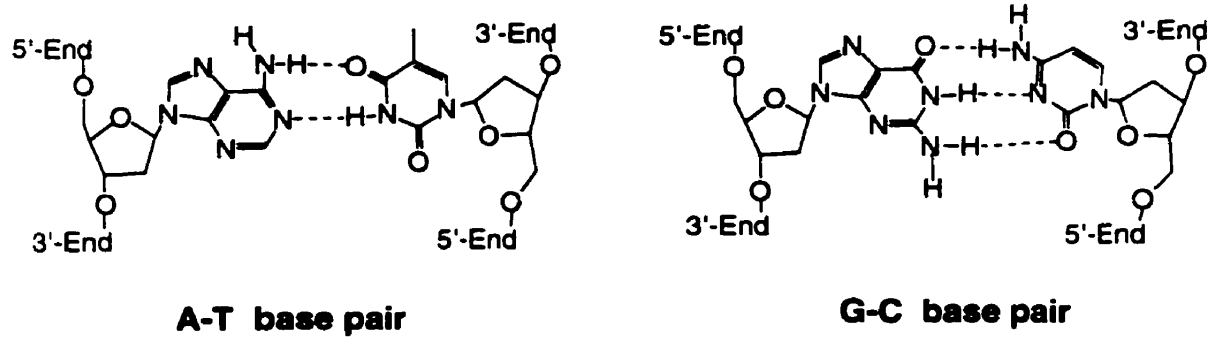


Figure 2. Watson-Crick Base Pairing

[4] Watson, J. S.; Crick, F. H., *Nature* **1953**, *171*, 737.

The antisense strategy appears to have many advantages. On transcription, every gene gives rise to a number of copies of messenger RNA, which are translated into a large number of protein molecules. Inhibition of gene expression is therefore more efficient than inhibition of the resulting protein product. Due to the ability of single-stranded nucleic acids to form double helices according to the rules of Watson-Crick base pairing, it is straightforward to design an unique oligonucleotide which should only bind to the target sequence of a single mRNA by forming a local duplex structure and thereby inhibit the synthesis of the corresponding protein. Antisense oligonucleotides can therefore be rationally designed.

The first instance of an antisense effect was reported in 1978 by Zamecnik and Stephenson.[5,6] They used a 13-mer unmodified oligonucleotide that was complementary to the RNA of Rous sarcoma virus to inhibit the growth of this virus in cell culture. Since then, antisense strategy has been widely studied, and several pharmaceutical companies are developing antisense drugs.[7,8]

Currently there are two ways to explain the antisense activities. The first one is the formation of a stable duplex between the antisense oligonucleotide and the mRNA that would simply keep the ribosome from binding to the mRNA at the target sequence, therefore inhibiting the translation from mRNA to protein. The second one, considered more likely in today's antisense agents, is that the ubiquitous nuclease RNase H will degrade the mRNA strand of a hybrid DNA:RNA duplex.[9] The degradation of the bound mRNA leads to rapid destruction of the encoded building plan for protein synthesis.

As a drug, the antisense oligonucleotide has to be able to penetrate through the cell membrane, and has to be stable to intracellular and extracellular enzymes. It also has to be specific to the target sequence, only inhibiting the information for disease related protein, and not interfering with the normal translation of genes.

Since, statistically, a 17-mer oligonucleotide occurs just once in the sequence of the entire human genome, the binding specificity can be achieved by choosing a long sequence

---

[5] Zamecnik, P. C.; Stephenson, M. L., *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 280.
[6] Stephenson, M. L.; Zamecnik, P. C., *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 285.
[7] Roush, W., *Science* **1997**, *276*, 1192.
[8] Rawls, R. L., *C&EN* **1997**, *6 (2)*, 35.
[9] Watson, J. D.; Hopkins, N. H.; Roberts, J. W.; Steitz, J. A.; Weiner, A. M., Molecular Biology of the Gene, 4th Ed., 1987, The Benjamin/Cummings Publishing Company, Inc. pp 298.

(>17-mer) oligonucleotide. Naturally occurring oligonucleotides (DNA and RNA) are unstable to nucleases. They are rapidly degraded by naturally occurring nucleases that hydrolytically cleave the phosphodiester backbone. In order to overcome these existing hurdles and to gain high hybridization of duplexes, chemically modified oligonucleotides were investigated.

1.2. Chemically Modified Oligonucleotides

DNA is a long, threadlike macromolecule made up of a large number of deoxyribonucleotides, each composed of a base, a sugar, and a phosphate group. The bases of DNA molecules carry genetic information, whereas their sugar and phosphate groups perform a structural role. Chemical modifications on natural DNA oligonucleotides can be done on all their constituents: base, sugar, and phosphate (backbone) residues, as shown in Figure 3. Most successful modifications improve the properties of oligonucleotides by increasing their nuclease resistance and RNA binding affinity.

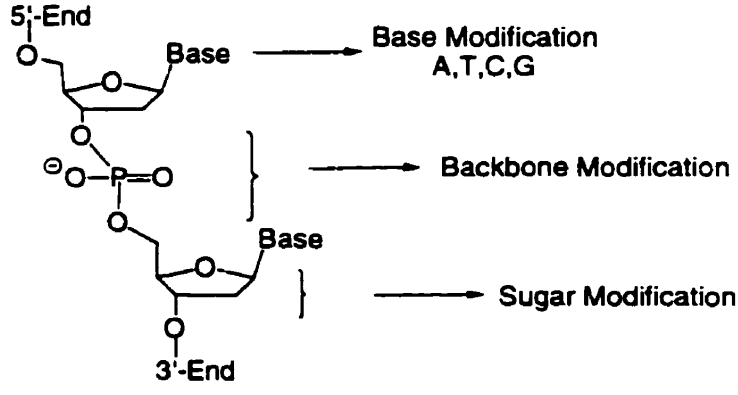


Figure 3. Chemical Modifications on Natural Oligonucleotide

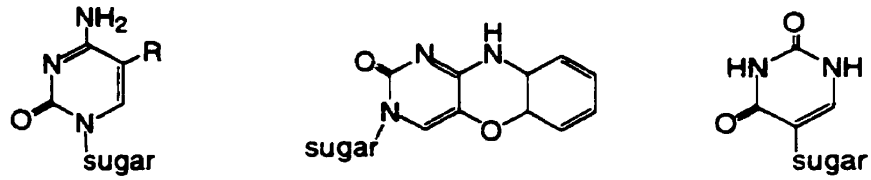
A large amount of modified oligonucleotides have been described in the literature, and there are several review articles.[10,11,12] The following is a concise summary.

---

[10] Uhlmann, E.; Peyman, A., *Chem. Rev.* **1990**, *90*, 543.
[11] De Mesmaeker, A.; Haner, R.; Martin, P.; Moser, H. E., *Acc. Chem. Res.* **1995**, *28*, 366.
[12] Herdewijn, P., *Liebigs Ann.* **1996**, 1337.

1.2.1. Base Modification

Base modification has a rather limited scope since the ability of Watson-Crick base pairing should not be disrupted. However, several modified bases revealed promising properties for a potential application in antisense oligonucleotides.[13] One successful modification of the bases was, for example, the substitution of cytosine with 5-methyl- or 5-bromocytosine **1** in oligodeoxyribonucleotides which resulted in increasing stability of DNA/RNA hybrids.[14]



1 R = -CH_3, -Br **2** **3**

Figure 4. Modified Bases

The scope of base modification has been extended to using non-natural heteroaromatic groups, such as **2**,[15] **3**,[16] which showed a dramatic stabilization of duplexes.

1.2.2. Sugar Modification

Besides the complementarity of bases, the configurational and conformational complementarity between antisense oligonucleotide and target segment of mRNA is also important for efficient hybridization. Most sugar modifications were aimed at conformationally restricting oligonucleotides so as to form a preorganized structure. In so doing, the hybridization process should benefit from less negative entropy changes during duplex formation.

---

[13] Sanghvi, Y. S., *Antisense Research and Applications*, Crooke, S. T., Lebleu, B., CRC Press, Inc.; Boca Raton, FL, **1993**, pp 273.
[14] Sanghvi, Y. S.; Hoke, G. D.; Freier, S. M.; Zounes, M. C.; Gonzalez, C.; Cummins, L.; Sasmor, H.; Cook, P. D., *Nucleic Acids Res.* **1993**, *21*, 3197.
[15] Lin, K.-Y.; Jones, R. J.; Matteucci, M., *J. Am. Chem. Soc.* **1995**, *117*, 3873.

$C_{3'}$-endo/N-pucker (A-RNA)

$C_{2'}$-endo/S-pucker (B-DNA)

Figure 5. Two Main Types of Sugar Conformations Found in A-RNA and B-DNA

RNA is found predominantly in the $C_{3'}$-endo conformation that is exclusively present in the A-form duplexes (Figure 5), probably due to a preferred gauche orientation of the 2'-substituent and the ring oxygen.[17] Short RNA/RNA duplexes are usually more stable than the DNA/RNA duplexes of the same sequence.[18] So one rational modification is to produce RNA like or $C_{3'}$-endo like oligonucleotides in order to beneficially influence the RNA binding behavior. Indeed, the incorporation of 2'-O-methyl ribonucleoside **4a**, 2'-O-allyl ribonucleoside **4b**,[19,20] 2'-methoxyethoxy nucleoside **4c**,[21,22] or 2'-fluoro-2'-deoxyribonucleoside **4d**[23] in oligonucleotides increases the affinity toward the RNA complement.

The conformation of the sugar has a great effect on the properties of oligonucleotides. It has been reported that the α-anomeric nucleosides **5** have a parallel

---

[16] Hall, K. B.; McLaughlin, L. W., *Biochemistry* **1991**, *30*, 1795.
[17] Saenger, W., Principles of Nucleic Acid Structure, Springer-Verlag, New York, Berlin, Heidelberg, Tokyo, **1984**.
[18] Freier, S. M., *Antisense Research and Applications*, Crooke, S. T., Lebleu, B., CRC Press, Inc.; Boca Raton, FL, **1993**, pp 67.
[19] Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E., *Nucleic Acids Res.* **1987**, *15*, 6131.
[20] Lesnik, E. A.; Guinosso, C. J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L. L.; Ecker, D. J.; Cook, P. D.; Freier, S. M., *Biochemistry* **1993**, *32*, 7832.
[21] Martin, P., *Helv. Chim. Acta.* **1995**, *78*, 486.
[22] 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.; Moser, H. E., *Chimia* **1996**, *50*, 168.
[23] Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzalez, C.; Cook, P. D., *J. Med. Chem.* **1993**, *36*, 831.

orientation to the target strand whilst increasing the duplex stability[24] and have high nuclease stability.[25]

4a R = OCH_3
4b R = OCH_2CHCH_2
4c R = $OCH_2CH_2OCH_3$
4d R = F

5

Figure 6. Modified Sugars

1.2.3. Backbone Modification

So far, the backbone modifications have been the most exploited class of variations since they retain the bases of DNA that are essential for binding and sequence-specificity, and the sugar that allows to orient the base with respect to the backbone axis. The efforts can be divided into phosphorus containing and phosphorus free backbone modifications.

On the phosphorus containing modified backbones, the phosphorus atom of the linkage was kept, only the substituent groups around the phosphorus were changed, as shown in Figure 7.

The phosphorothioates **6** are the most advanced candidates in clinical trials.[26] Methylphosphonates **7**,[27] phosphorodithioates **8**,[28] phosphotriester **9**,[29] phosphoramidates **10**,[30,31] **11**,[32] boranophosphates **12**,[33] and phosphorofluoridates **13**[34] have all been

---

[24] Gagnor, C.; Bertrand, J.-R.; Thenet, S.; Lemaitre, M.; Morvan, F.; Rayner, B.; Malvy, C.; Lebleu, B.; Imbach, J.-L.; Paoletti, C., *Nucleic Acids Res.* **1987**, *15*, 10419.
[25] Morvan, F.; Rayner, B.; Imbach, J.-L.; Thenet, S.; Bertrand, J.-R.; Paoletti, J.; Malvy, C.; Paoletti, C., *Nucleic Acids Res.* **1987**, *15*, 3421.
[26] Matsukura, M.; Shinozuka, K.; Zon, G.; Mitsuya, H.; Reitz, M., Cohen, J.S.; Broder, S., *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 7706.
[27] Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'O, P. O. P., *Biochemistry* **1979**, *18*, 5134.
[28] Marshall, W. S.; Caruthers, M. H., *Science* **1993**, *259*, 1564.
[29] Miller, P. S.; Fang, K. N.; Kondo, N. S.; Ts'O, P. O. P., *J. Am. Chem. Soc.* **1971**, *93*, 6657.
[30] Letsinger, R. L.; Singman, C. N.; Histand, G.; Salunkhe, M. J., *J. Am. Chem. Soc.* **1988**, *110*, 4470.

synthesized and tested as possible antisense agents. All the oligonucleotides incorporated with these backbones showed better resistance towards exonucleases and endonucleases.

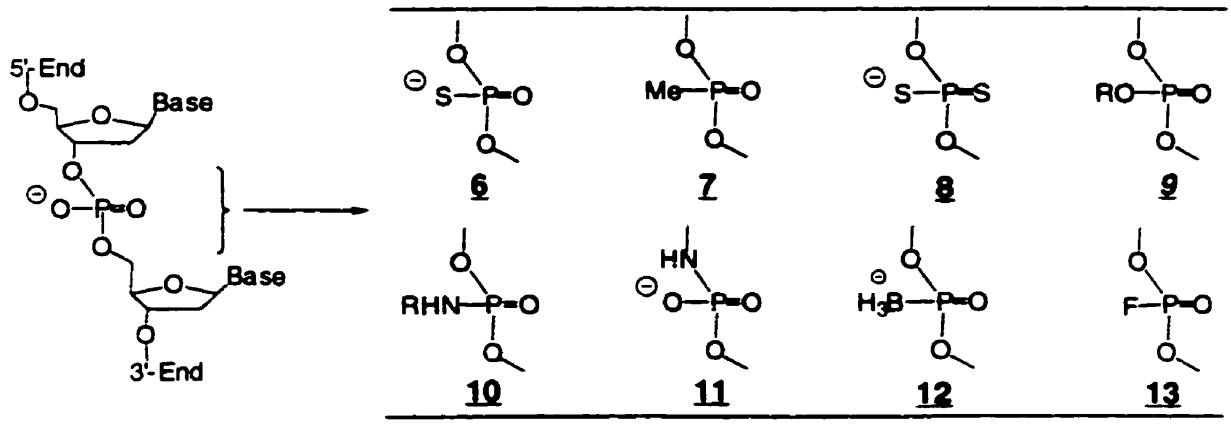


Figure 7. The Phosphorus Containing Backbone Modification

The phosphorus free backbone modifications focused on non-phosphorus internucleoside linkages.[35] Some of these dephospho linkages are shown in Figure 8.

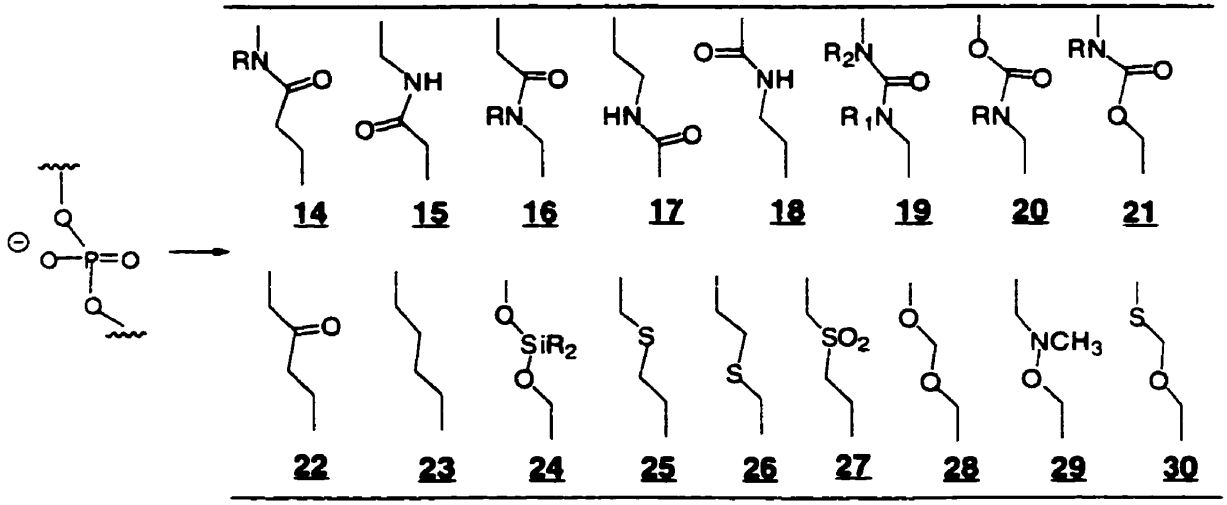


Figure 8. The Phosphorus Free Backbone Modification

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[31] Ozaki, H.; Yamoto, S.; Maikuma, S.; Honda, K.; Shimidzu, T., *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3869.
[32] Gryaznov, S.; Chen, J.-K., *J. Am. Chem. Soc.* **1994**, *116*, 3143.
[33] Sood, A.; Shaw, B. R.; Spielvogel, B. F., *J. Am. Chem. Soc.* **1990**, *112*, 9000.
[34] Dabkowski, W.; Cramer, F.; Michalski, J., *Tetrahedron Lett.* **1987**, *28*, 3561.
[35] For reviews, see reference 10, 11, 12 and Sanghvi, Y.S.; Cook, P.D. Towards the second-generation synthetic backbones for antisense oligonucleotides. *In Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C.K., Baker, D. C., Eds.; Plenum Press: New York, **1993**; pp 311.

As expected, these backbone replacements greatly increased the nuclease resistance of corresponding oligonucleotides. However, most of these modifications, such as amide **14**,[36] **17**,[37] **18**,[38] urea **19**,[39] carbamates **20** and **21**,[40] decrease the binding affinity to the RNA compared with natural oligonucleotides. Only several exceptions, amides **15**[41,42] and **16**,[43,44] the N-methylhydroxylamine **29**,[45] and the thioformacetal **30**[46] displayed equivalent or even slightly increased RNA binding properties. The synthesis of these dephospho analogues often involved many steps and few if any oligomers incorporating exclusively these bridges have been made.

Recently, the peptide nucleic acids (PNAs) which involve the replacement of both the sugar and the backbone by an amide chain, as shown in Figure 9, were also reported to be good binders of RNA and DNA.[47,48,49]

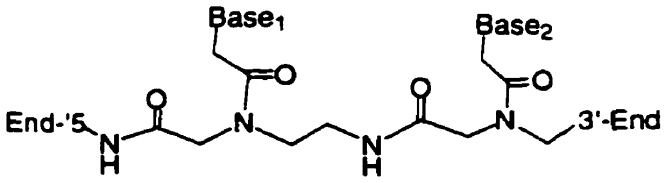

Figure 9. Peptide Nucleic Acids (PNAs)

1.2.4. Other Modifications

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[36] Lebreton, J.; De Mesmaeker, A.; Waldner, A.; Fritsch, V.; Wolf, R.M.; Freier, S.M., *Tetrahedron Lett.* **1993**, *34*, 6383.
[37] De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M., *Synlett* **1993**, 733.
[38] De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M., *Bioorg. Med. Chem. Lett.* **1994**, 4, 873.
[39] Waldner, A.; De Mesmaeker, A.; Lebreton, J.; Fritsch, V.; Wolf, R. M., *Synlett.* **1994**, 57.
[40] Waldner, A.; De Mesmaeker, A.; Lebreton, J., *Bioorg. Med. Chem. Lett.* **1994**, *4*, 405.
[41] Lebreton, J.; Waldner, A.; Lesueur, C.; De Mesmaeker, A., *Synlett.* **1994**, 137.
[42] De Mesmaeker, A.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R. M., Freier, S. M., *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 226.
[43] Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; De Mesmaeker, A., *Tetrahedron Lett.* **1994**, *35*, 5225.
[44] Idziak, I.; Just, G.; Damha, M. J.; Giannaris, P. *Tetrahedron Lett.* **1993**, *34*, 5417.
[45] Vasseur, J. J.; Debart, F.; Sanghvi, Y. S.; Cook, P. D., *J. Am. Chem. Soc.* **1992**, *114*, 4006.
[46] Jones, R. J.; Lin, K-Y.; Milligan, J. F.; Wadwani, S.; Matteucci, M. D. *J. Org. Chem.* **1993**, *58*, 2983.
[47] Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895.
[48] Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Science* **1991**, *254*, 1497.
[49] Nielsen, P.E.; Egholm, M.; Buchardt, O. *Bioconjugate Chem.* **1994**, *5*, 3.

We have seen that there are many ways to improve the properties of antisense oligonucleotides, such as increased binding affinity and nuclease resistance. These properties also can be improved by substitution on oligonucleotides with relatively simple molecules. Oligonucleotides have been modified with a large number of chemical groups such as intercalators, hydrophobic residues, alkylating groups, or other chemically reactive molecules.

The intercalating groups can greatly increase the binding affinity of an oligonucleotide. The commonly used intercalator are derivatives of acridine,[50] anthraquinone,[51,52] and pyrene.[53] Hydrophobic groups are linked to oligonucleotides to improve cellular uptake. Substitution of antisense oligonucleotides with cholesterol,[54] cholic acid,[55] or long aliphatic alkyl chains,[56,57] has a pronounced effect on cellular uptake and antiviral efficiency. Oligonucleotides bearing chemically reactive groups can be used for sequence specific modification and/or cleavage of targeted nucleic acids.[58] Alkylating group[59] and cross-linking agents such as aryl azide,[60] porphyrine,[61] psoralene[62,63] have been used for sequence specific binding purpose. Metal complexes attached to oligonucleotides have been widely used for the specific cleavage of nucleic acids.[64,65]

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[50] Helene, C. *Genome* **1989**, *31*, 413.
[51] Keller, T. H.; Haner, R., *Nucleic Acids Res.* **1993**, *21*, 4499.
[52] Mori, K.; Subasinghe, C.; Cohen, J. S. *FEBS Lett.* **1989**, *249*, 213.
[53] Yamana, K.; Letsinger, R. L. *Nucleic Acids Res.* **1985**, *16*, 169.
[54] Svinarchuk, F. P.; Konevetz, D. A.; Pliasunova, O. A.; Pokrovsky, A. G.; Vlassov, V. V. *Biochimie* **1993**, *75*, 49.
[55] Manoharan, M.; Johnson, L. K.; Bennett, C. F.; Vickers, T. A.; Ecker, D. J.; Cowsert, L. M.; Freier, S. M.; Cook, P. D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1053.
[56] Kabanov, A. V.; Vinogradov, S. V.; Ovcharenko, A. V.; Krivonos, A. V.; Melik-Nubarov, N. S.; Kiselev, V. I.; Severin, E. S. *FEBS Lett.* **1990**, *259*, 327.
[57] Shea, R. G.; Marsters, J. C.; Bischofberger, N. *Nucleic Acids Res.* **1990**, *18*, 3777.
[58] Knorre, D. G.; Vlassov, V. V. *Nucleic Acids Res.* **1985**, *32*, 291.
[59] Boutorine, A. S.; Boiziau, C.; Le Doan, T.; Toulme, J. J.; Helene, C. *Biochimie* **1992**, *74*, 485.
[60] Levina, A. S.; Berezovskii, M. V.; Venjaminova, A. G.; Dobrikov, M. I.; Repkova, M. N.; Zarytova, V. F. *Biochimie* **1993**, *75*, 25.
[61] Ortigao, J. F. R.; Ruck, A.; Gupta, K. C.; Rosch, R.; Steiner, R.; Seliger, H. *Biochimie* **1993**, *75*, 29.
[62] Pieles, U.; Sproat, B. S.; Neuner, P.; Cramer, F. *Nucleic Acids Res.* **1989**, *17*, 8967.
[63] Kean, J. M.; Murakami, A.; Blake, K. R.; Cushman, C. D.; Miller, P. S. *Biochemistry* **1988**, *27*, 9113.
[64] Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.
[65] Chin, J. *J. Acc. Chem. Res.* **1991**, *24*, 145.

1.3. Oligonucleotide Phosphorothioates and Methylphosphonates

1.3.1. Oligonucleotide Phosphorothioates

Oligonucleotide phosphorothioates (OPS) have been the most extensively studied antisense oligonucleotides so far. Their antiviral effects were described back in 1970 by De Clercq and Eckstein.[66] In OPS, one of the phosphate oxygen atoms not involved in the bridge is replaced by a sulfur atom, with the negative charge being distributed unsymmetrically and located mainly on sulfur.[67,68] This substitution results in properties such as stability to nucleases and retention of solubility in water, which makes it exceptionally interesting for use in antisense technology.

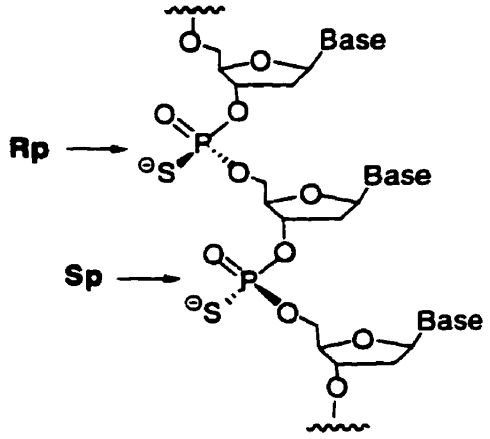

Figure 10. Two Diastereomers of Phosphorothioate Linkage

One other important property for OPS is that they are accepted as substrates by RNase H to induce cleavage of the bound RNA strand. RNase H which naturally serves the purpose to remove RNA primers during DNA replication, is highly sensitive to structural changes within the antisense strand, and only a few modifications such as phosphorothioates,[69] or phosphorodithioates[70] are known to be accepted.

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[66] De Clercq, E.; Eckstein, F.; Sternbach, H.; Morgan, T. C. *Virology* **1970**, *42*, 421.
[67] Frey, P. A.; Sammons, R. D. *Science* **1985**, *228*, 541.
[68] Iyengar, R.; Eckstein, F.; Frey, P. A. *J. Am. Chem. Soc.* **1984**, *106*, 8309.
[69] Mirabelli, C. K.; Bennett, C. F.; Anderson, K.; Crooke, S. T. *Anti-Cancer Drug Des.* **1991**, *6*, 647.
[70] Marshall, W. S.; Caruthers, M. H. *Science* **1993**, *259*, 1564.

OPS are currently the most active oligonucleotide derivatives for anti-HIV activity. They inhibit directly not only HIV reverse transcriptase but also cellular DNA polymerases α and γ.[71]

OPS by now are the only antisense oligonucleotides which have been on clinical studies. Table 1 shows several products developed by ISIS Pharmaceuticals company.[72]

Table 1. Antisense Oligonucleotides Developed by ISIS Pharmaceuticals

Compound	Target	Disease	Status
Fomivirsen	HCMV	Retinitis (AIDS)	Phase III
ISIS 2302	ICAM-1	Crohn's Disease	Pivotal Trial
		Rheumatoid Arthritis	Phase II
		Psoriasis	Phase II
		Renal Transplant Rejection	Phase II
ISIS 2302	PKC-alpha	Cancer	Phase II
ISIS 5132	c-raf kinase	Cancer	Phase II
ISIS 5320	HIV	AIDS	Phase I Completed
ISIS 2503	Ha-ras	Cancer	Phase I
ISIS 13312	HCMV	Retinitis (AIDS)	IND Candidate

However, an unsolved and often unappreciated problem concerning the use of OPS in the antisense strategy is their polydiastereoisomerism. Replacement of one of the two nonbridging oxygens at phosphorus by sulfur induces asymmetry at the phosphorus atom (Figure 10). Considering a 20-mer antisense oligonucleotide, the number of possible diastereomers would be 2^{19}, half a million different molecules.

Since molecular recognition is often dependent on the chirality of the substrate,[73] one may reasonably hypothesize that the biological activity of the OPS diastereomers may also be stereodependent. For example, OPS possessing only Rp linkages were found to be resistant to endonucleases P1, whereas the Sp oligonucleotides were all cleaved under the

[71] Majumdar, C.; Stein, C. A.; Cohen, J. S.; Broder, S.; Wilson, S. H. *Biochemistry* **1989**, *28*, 1340.
[72] From Internet: Http://www.isip.com

same conditions.[74] On the other hand, snake venom phosphodiesterase digested only terminal nucleotides having the Rp configuration.[75] Therefore, it is important, from a theoretical and a practical point of view, to develop a methodology for stereoselective synthesis of OPS. Since the methods for diastereoselective synthesis of phosphorothioates by now are limited in scope, this project also presents a synthetic challenge.

1.3.2. Oligonucleotide Methylphosphonates

Oligodeoxyribonucleoside methylphosphonates (OMP) contain a non ionic (3'-5') internucleotide methylphosphonate link in place of the naturally occurring phosphodiester internucleotide bond. These analogues are particularly attractive as antisense reagents because of their nuclease resistance and their ability to be taken up intact by mammalian cells.[76]

The OMP are resistant to hydrolysis by purified exo- and endonucleases and by the nucleases found in bovine and human serum. Oligothymidylates having alternating methylphosphonate-phosphodiester linkages are completely resistant to cleavage by spleen phosphodiesterase and S_1 endonuclease. The phosphodiester linkages of these oligomers are slowly hydrolyzed by snake venom phosphodiesterase and by micrococcal nuclease.[77] In addition to being resistant to nuclease degradation, OMP are resistant to hydrolysis by RNase H,[78] and are stable to *Escherichia coli* DNA polymerase I and calf thymus DNA polymerase.[79]

OMP are taken up intact by mammalian cells in culture. The kinetics of uptake by transformed Syrian hamster fibroblasts of OMP with chain lengths from three to nine are essentially the same.[80] OMP 18 and 21 nucleotides in length have been reported to be taken

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[73] Konig, B. *J. Prakt. Chem.* **1995**, 339.
[74] Griffiths, A. D.; Potter, B. V. L.; Eperon, I. C. *Nucleic Acids Res.* **1987**, *15*, 4145.
[75] Burgers, P. M. J.; Eckstein, F.; Hunneman, D. H. *J. Biol. Chem.* **1979**, *254*, 7.
[76] Murray, J. A. H. Antisense RNA and DNA, John Wiley and Sons, Inc. **1992**, pp 241.
[77] Miller, P. S.; Dreon, N.; Pulford, S. M.; McParland, K. B. *J. Biol. Chem.* **1980**, *235*, 9659.
[78] Quartin, R.; Brakel, C.; Wetmur, J. *Nucleic Acids Res.* **1989**, *17*, 7253.
[79] Miller, P. S.; Annan, N. D.; McParland, K. B.; Pulford, S. M. *Biochemistry* **1982**, *21*, 2507.
[80] Miller, P. S.; McParland, K. B.; Jayaraman, K.; Ts'o, P. O. P. *Biochemistry*, **1981**, *20*, 1874.

up in a concentration dependent manner respectively by CV-1 cells and daunorubicin-resistant K562/III cell cultures.[81,82]

Antisense OMP can inhibit protein synthesis in both bacterial and mammalian cell-free systems and in cells in culture in a sequence-specific manner.[83] They have been targeted against the functional regions of bacterial ribosomal RNA (rRNA)[84] and mammalian mRNA,[85,86] as well as against splice junction regions of precursor mRNA.[87,88] It was reported that OMP can inhibit the activity of human collagenase.[89]

These nuclease resistance and sequence specific antisense activities of OMP made them very attractive to be developed as highly selective antiviral and chemotherapeutic antisense agents.

As OPS, OMP suffer the same problem. Changing one of two nonbridging oxygens at phosphorus with methyl group affords two diastereomers, as shown in Figure 11.

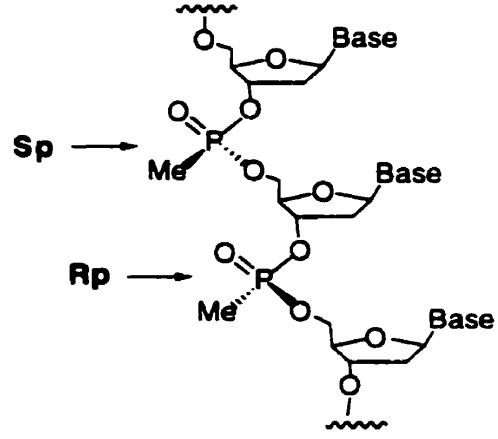

Figure 11. Two Diastereomers of Oligonucleotide Methylphosphonates

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[81] Marcus-Sekura, C. J.; Woerner, A. M.; Shinozuka, K.; Zon, G.; Quinnan, Jr., G. V. *Nucleic Acids Res.* **1987**, *15*, 5749.
[82] Vasanthakumar, G.; Ahmed, N. K. *Cancer Comm.* **1989**, *1*, 225.
[83] Miller, P. S. *BioTechnology* **1991**, *9*, 358.
[84] Jayaraman, K.; McParland, K.; Miller, P.; To's, P. O. P. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 1537.
[85] Blake, K. R.; Murakami, A.; Spitz, S. A.; Glave, S. A.; Reddy, M. P.; Ts'o, P. O. P.; Miller, P. S. *Biochemistry* **1985**, *24*, 6139.
[86] Kean, J. M.; Murakami, A.; Blake, K. R.; Cushman, C. D.; Miller, P. S. *Biochemistry* **1988**, *27*, 9113.
[87] Smith, C. C.; Aurelian, L.; Reddy, M. P.; Miller, P. S.; Ts'o, P. O. P. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 2787.
[88] Zaia, J. A.; Rossi, J. J.; Murakawa, G. J.; Spallone, P. A.; Stephens, D. A.; Kaplan, B. E.; Eritja, R.; Wallace, R. B.; Cantin, E. M. *J. Virol.* **1988**, *62*, 3914.
[89] Delong, R. K.; Miller, P. S. *Antisense & Nucleic Acid Drug Development* **1996**, *6*, 273.

The conformations of the two diastereomers of dinucleoside methylphosphonates have been studied.[90] The stacking interactions and the sugar conformation of these dimers are very similar to those of the corresponding natural dinucleoside monophosphates. The Sp diastereomer in d-ApA has the same conformations as the natural one, whereas the Rp isomer shows less base-base stacking interaction.[91] The position and configuration of a single methylphosphonate linkage affected the stability of duplex. Duplexes containing methylphosphonate groups with the Rp-Rp configuration generally were more stable than those containing Sp-Sp methylphosphonate linkages. Proton NMR nuclear Overhauser effect (NOE) measurements indicated that the Sp-methyl group interacts with the H-3' on the adjacent ribose residue in the duplex.[92] This steric interaction could contribute, in part, to lower stability of the duplex. Reynolds[93] and Stec[94] reported that OMP with Rp configuration bind RNA with significantly higher affinity than these Sp diastereomers. Therefore, the stereospecific synthesis of methylphosphonates is crucial for the development of these OMP into antisense agents.

1.4. Stereoselective Synthesis of Phosphorothioates

Oligonucleotides are most commonly synthesized on solid phase using the phosphoramidite method developed by McBride and Caruthers.[95,96] The procedure involves reacting the 5'-end of an (oligo)nucleotide **34** attached to the solid support[97] with an excess of 5'-protected phosphoramidite **31**, as shown in Figure 12. The reaction,

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[90] Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'o, P. O. P. *Biochemistry* **1979**, *18*, 5134.
[91] Kan, L. S.; Cheng, D. M.; Miller, P. S.; Yano, J.; Ts'o, P. O. P. *Biochemistry* **1980**, *19*, 2122.
[92] Bower, M.; Summers, M. F.; Powell, C.; Shinozuka, K.; Regan, J. B.; Zon, G.; Wilson, W. D. *Nucleic Acids Res.* **1987**, *15*, 4915.
[93] Reynolds, M. A.; Hogrefe, R. I.; Jaeger, J. A.; Schwartz, D. A.; Riley, T. A.; Marvin, W. B.; Daily, W. J.; Vaghefi, M. M.; Beck, T. A.; Knowles, S. K.; Klem, R. E.; Arnold, L. J., *Nucleic Acids Res.*, **1996**, *24*, 4584.
[94] Stec, W. J.; Wilk, A. *Angew. Chem. Intl. Ed.* **1994**, *33*, 709.
[95] McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245.
[96] Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223.
[97] Kumar, P.; Sharma, A. K.; Sharma, P.; Garg, B. S.; Gupta, K. C. *Nucleosides & Nucleotides* **1996**, *15*, 879.

catalyzed by tetrazole, is complete within minutes and provides a phosphite triester **32** in nearly quantitative yield.[98] After capping unreacted nucleosides, oxidation with I_2/H_2O gives the desired phosphate triester **33**. Repeating the procedure n-1 times, a n-mer oligonucleotide is obtained. At the end, a treatment with ammonium hydroxide allows for the removal of the protective groups from the bases, elimination of the cyanoethyl protective groups on the phosphate diester bridges, and cleavage of the n-mer oligonucleotide **35** from the solid support after detritylation.

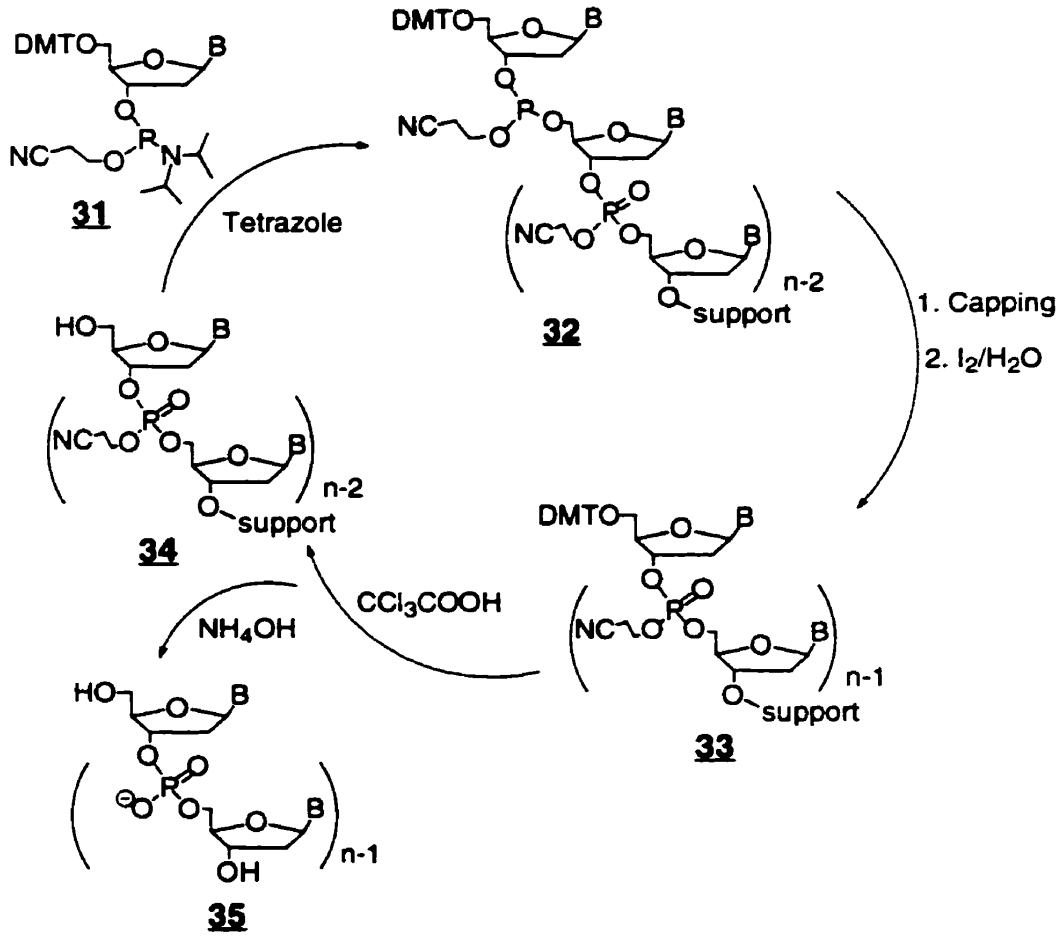

Figure 12. The Synthesis of Oligonucleotide on Solid Phase

[98] Caruthers, M. H. DNA Synthesis for Nonchemists: The Phosphoramidite Method on Solid Supports. In Synthesis and Applications of DNA and RNA; Narang, S. A., Ed.; Academic Press, Inc.; Orlando, **1987**. pp 47.

This synthetic strategy was easily adapted to the synthesis of phosphorothioate derivatives or of mixed phosphate-phosphorothioates sequences.[99,100] Only one step that differs from the above procedure is the oxidation step, which has to be replaced by a sulfurization step. Different sulfurizing reagents have been developed and assessed.[101] Currently Beaucage's reagent (3H-1,2-benzodithiol-3-one 1,1-dioxide) is commonly used on DNA synthesizers as the sulfurizing reagent.[102]

However, in this procedure, even starting with a chiral phosphoramidite **36**, the stereochemistry at the phosphorus atom is not controlled and the resulting product **38** turns out to be a mixture of two diastereomers.[103]

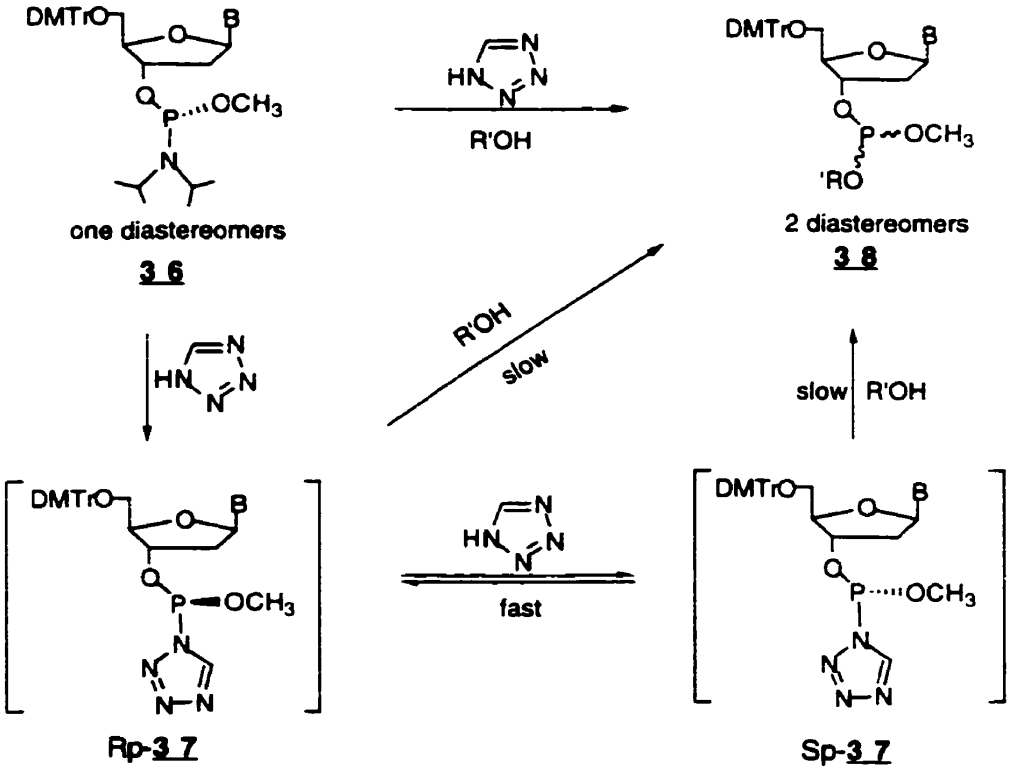

Figure 13. The Mechanism of Epimerization Caused by Tetrazole

The mechanism of epimerization caused by tetrazole proposed by Stec is shown in Figure 13. Later this mechanism was proven by Berner. [104,105] Thus, the present DNA

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[99] Stec, W. J.; Zon, G.; Egan, W.; Stec, B. *J. Am. Chem. Soc.* **1984**, *106*, 6077.
[100] Connolly, B. A.; Potter, B. V. L.; Eckstein, F.; Pingoud, A.; Grotjahn, L. *Biochemistry* **1984**, *23*, 3443.
[101] Cheruvallath, Z. S.; Cole, D. L.; Ravikumar, V. T. *Nucleosides & Nucleotides* **1996**, *15*, 1441.
[102] R. P. Iyer, W. Egan, J. B. Regan, and S. L. Beaucage, *J. Am. Chem. Soc.* **1990**, *112*, 1253.

synthesizer cannot be used to synthesize chiral phosphorothioates. Many groups have tried to develop methodologies for the stereoselective synthesis of phosphorothioates. The following is a summary for their methods.

1.4.1. Enzymatic Synthesis

One approach to the stereoselective synthesis of phosphorothioates is to use enzymes. Since polymerases, transferases and nucleases have the ability to assist in the synthesis or degradation of phosphorothioates in a stereo-defined manner, all these enzymes have been considered.

The first enzymatic stereocontrolled synthesis of phosphorothioates was reported by Eckstein and co-workers.[106] They used DNA-dependent RNA polymerase from *E. Coli* and synthesized oligonucleotide phosphorothioates having the Rp configuration (Figure 14.)[107,108,109]

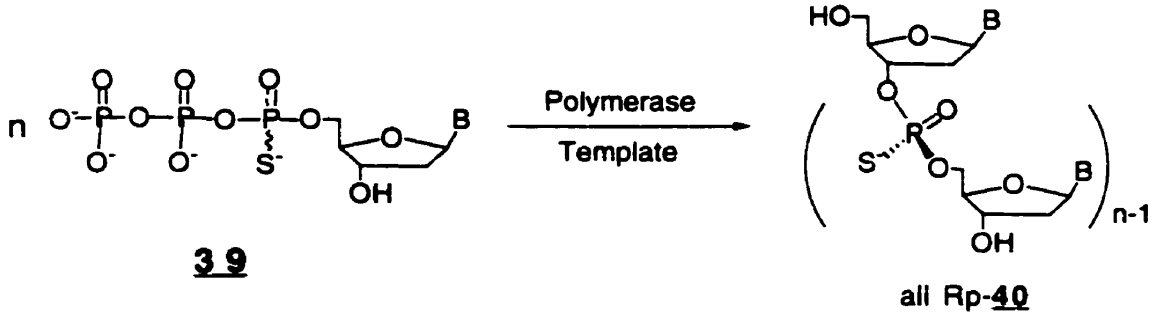

Figure 14. The Enzymatic Synthesis of Phosphorothioates

Later, several other polymerases were found to be able to catalyze the stereospecific formation of oligonucleotide phosphorothioates having consistently the Rp configuration, such as DNA-dependent DNA polymerases from *E. Coli*,[110] Phage T4,[111] Phage T7,[112]

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[103] Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279.
[104] Berner, S.; Muhlegger, K.; Seliger, H. *Nucleoside & Nucleotides* **1988**, *7*, 763.
[105] Berner, S.; Muhlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853.
[106] Matzura, H.; Eckstein, F. *Eur. J. Biochem.* **1968**, *63*, 448.
[107] Eckstein, F.; Gindl, H. *Eur. J. Biochem.* **1970**, *13*, 558.
[108] Eckstein, F.; Armstrong, V. W.; Sternbach, H. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 2987.
[109] Burgers, P. M. J.; Eckstein, F. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 4795.
[110] Burgers, P. M. J.; Eckstein, F. *J. Biol. Chem.* **1979**, *254*, 6889.
[111] Romaniuk, P. J.; Eckstein, F. *J. Biol. Chem.* **1982**, *257*, 7684.

Micrococcus luteus,[113] from polynucleotide phosphorylase,[114] tRNA nucleotidyl transferase,[115] RNA Ligase[116] and 2'-5'-oligoadenylate synthetase.[117,118] The limitation for this enzymatic synthesis is that all these enzymes lead to the Rp configuration.

Endonucleases for diastereoselective degradation of phosphorothioates having undesired Rp or Sp configurations have been studied, but they are limited in practice to short homopolymers.[119,120] For example, endonuclease P1 selectively cleaves all phosphorothioate diesters with Sp configuration.[121] Diastereoselective degradation of OPS with the undesired configuration is limited to phosphorothioates with mainly Rp or Sp configuration. Otherwise, diastereoselective enzymatic digestion would be impractical because the yield would be dramatically low.

1.4.2. Stereoselective Synthesis of Phosphorothioates from H-Phosphonates

Phosphorothioates also can be synthesized *via* H-phosphonate approach, which involves a coupling reaction of 5'-O-DMT-nucleoside 3'-O-(H-phosphonate) **41** with 5'-hydroxy nucleoside **42**, as shown in Figure 15. Activation is achieved with pivaloyl or adamantoyl chloride to form H-phosphonate **43**.[122,123] Sulfurization of **43** gave phosphorothioate **44**, and this sulfurization step is stereoretentive. Seela and Kretschmer separated the two diastereomers of H-phosphonate **43** by chromatography. They obtained stereo-pure dinucleotide phosphorothioate **44** after sulfurizing the diastereomerially pure H-phosphonate **43**.[124]

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[112] Brody, R. S.; Adler, S.; Modrich, P.; Stec, W. J.; Lesnikowski, Z. J.; Frey, P. A. *Biochemistry* **1982**, *21*, 2570.
[113] Eckstein, F.; Jovin, T. M. *Biochemistry* **1983**, *22*, 4546.
[114] Burgers, P. M. J.; Eckstein, F. *Biochemistry*, **1979**, *18*, 450.
[115] Eckstein, F.; Sternbach, H.; von der Haar, F. *Biochemistry* **1977**, *16*, 3429.
[116] Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1982**, *21*, 5877.
[117] Suhadolnik, R. J.; Choongeun, L. *Biochemistry* **1985**, *24*, 551.
[118] Kariko, K.; Sobol, R. W., Jr; Suhadolnik, L.; Li, S.-W.; Reichenbach, N. L.; Suhadolnik, R. J.; Charubala, R.; Pfleiderer, W. *Biochemistry* **1987**, *26*, 7127.
[119] Stec, W. J. *Nucleic Acids Symp. Ser.* **1991**, *23*, 171.
[120] Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, *19*, 5883.
[121] Griffiths, A. D.; Potter, B. V. L.; Eperon, I. C. *Nucleic Acids Res.* **1987**, 4145.
[122] Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. *Chem. Scr.* **1985**, *25*, 280.
[123] Andrus, A.; Efcavitch, J. W.; McBride, L.; Giusti, B. *Tetrahedron Lett.* **1988**, *29*, 861.
[124] Seela, F.; Kretschmer, U. *J. Org. Chem.* **1991**, *56*, 3861.

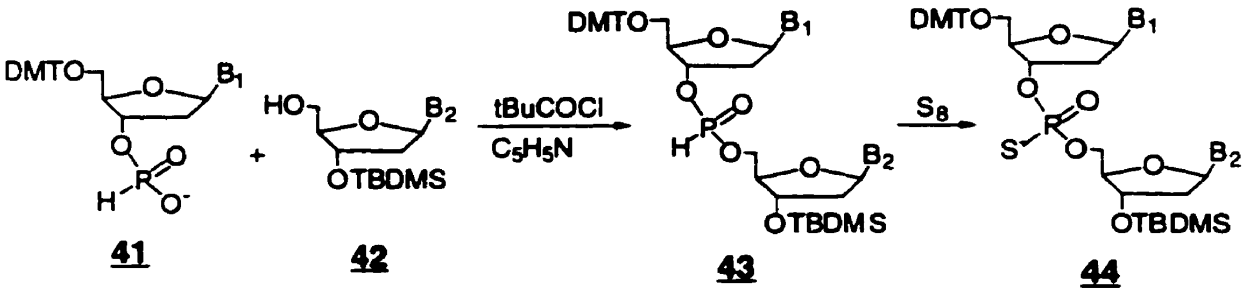

Figure 15. Synthesis of Phosphorothioate *via* H-Phosphonate

A stereocontrolled synthesis of H-phosphonate was reported by Hata and co-workers.[125] They found that degradation of acylphosphonate **45** with n-butylamine and DBU is stereoselective. Without purification, intermediate H-phosphonates **46** were sulfurized with element sulfur to give pure Rp-dinucleoside phosphorothioates. When the 3'-terminal group 1,3-benzodithiol-yl (BDT) on **45** was replaced by other groups, the reaction was not stereoselective.[126] A mechanism involving a nucleophilic attack of DBU to form a bipyramidal pentacoordinated phosphorus intermediate was proposed. Recently a similar intermediate was isolated by Merckling and Ruedi.[127]

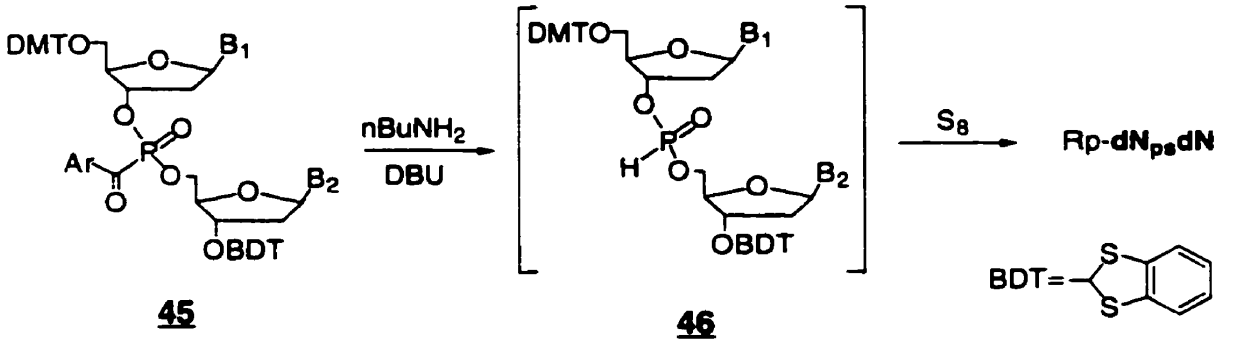

Figure 16. Stereocontrolled Synthesis of Phosphorothioates by Hata et al.

The other example for the sterically demanding protective groups was reported by Battistini et al.[128,129] Condensation of 2'-H-phosphonate nucleoside **47** protected on the 3'

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[125] Fujii, M.; Ozaki, K.; Kume, A.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1986**, *26*, 935.
[126] Fujii, M.; Ozaki, K.; Sekine, M.; Hata, T. *Tetrahedron.* **1987**, *43*, 3395.
[127] Merckling, F. A.; Ruedi, P. *Tetrahedron Lett.* **1996**, *37*, 2217.
[128] Battistini, C.; Brasca, M. G.; Fustinoni, S. *Nucleosides & Nucleotides* **1991**, *10*, 723.
[129] Battistini, C.; Brasca, M. G.; Fustinoni, S.; Lazzari, E. *Tetrahedron* **1992**, *48*, 3209.

and 5'-positions with 2',3'-protected nucleoside **48** provides exclusively Sp-configurated 2',5'-phosphorothioate **49**.

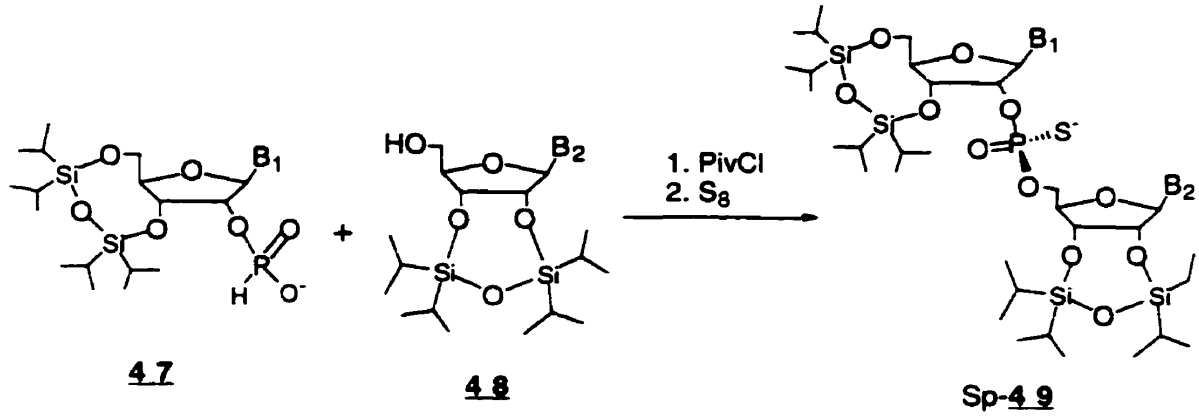

Figure 17. The Stereocontrolled Synthesis of Phosphorothioates by Battistini et al.

Changing the protecting group on **48** caused a reduction in diastereoselectivity. The authors rationalized the high diastereoselectivity in terms of the large steric hindrance created by the protective groups.

The limitation for these approaches is that they need special protecting groups, so they are not applicable to the synthesis of longer oligomers.

1.4.3. Stereocontrolled Nucleophilic Displacement at Tetracoordinated Phosphorus Centers

Lesnikowski et al. first reported the highly diastereoselective substitution of a p-nitrophenoxy group on tetracoordinated phosphorus compound **50** by a 5'-hydroxyl-nucleoside **51**.[130,131,132] The coupling reaction occurs with inversion of configuration at the phosphorus with a diastereoselectivity higher than 95% and an overall 70% yield. The limitation for this approach is that the chiral precursor **50** was separated from its two diastereomers by chromatography.

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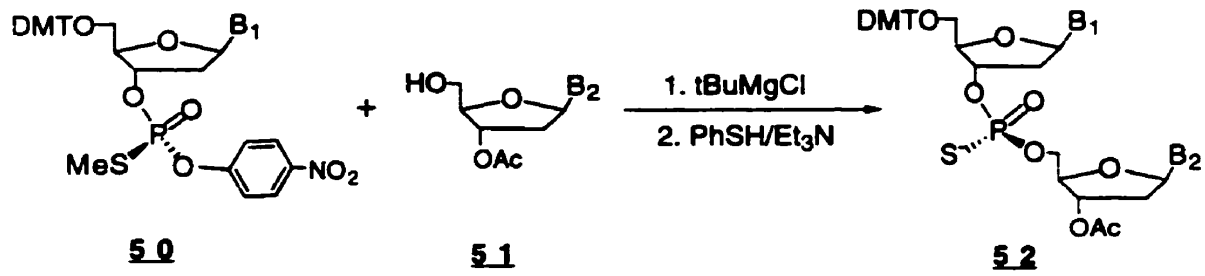
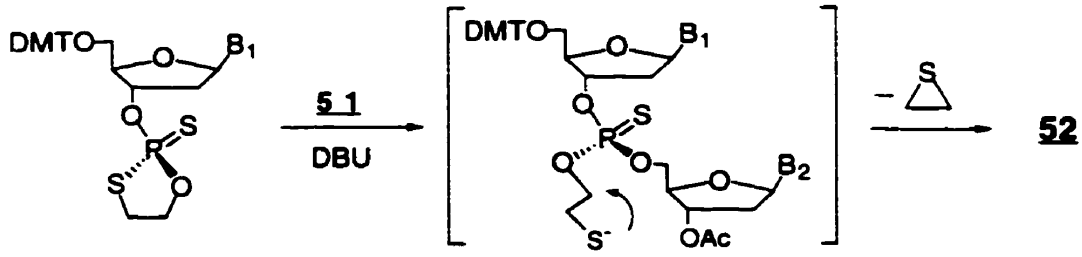
[130] Lesnikowski, Z. J.; Sibinska, A. *Tetrahedron* **1986**, *42*, 5025.
[131] Lesnikowski, Z. J.; Jaworska, M. *Tetrahedron Lett.* **1989**, *30*, 3821.
[132] Lesnikowski, Z. J. *Nucleosides & Nucleotides* **1992**, *11*, 1621.



50 51 52

Figure 18. Stereocontrolled Synthesis of Phosphorothioates by Lesnikowski et al.

Later, Stec and coworkers developed an oxathiaphospholane method, which can be used in solid phase synthesis.[133,134] In the presence of DBU, the oxathiaphospholane derivative **53** reacted with 3'-protected nucleoside to give chiral phosphorothioates **52** *via* intermediate **54**.



53 54

Figure 19. Stereocontrolled Synthesis of Phosphorothioates by Stec et al.

However, this method still suffers from the fact that the chiral precursors **53** have to be separated chromatographically.

1.4.4. Chiral Cyclic Phosphoramidites Method

Just and co-workers developed a cyclic phosphoramidite method.[135,136] A chirally pure phosphoramidite precursor **56** was prepared by incorporating the nitrogen,

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[133] Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, *19*, 5883.
[134] 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.
[135] Xin, Z.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 969.
[136] Xin, Z. Master's Thesis, McGill University, **1994**.

phosphorus, and oxygen atoms in a six-membered oxazaphosphorine ring derived from a chiral γ-aminoalcohol **55**. And a certain degree of stereocontrol was achieved in the coupling reaction of **56** with an alcohol by using substituted imidazole **57** as a catalyst. The two diastereomers **58** could be obtained as high a ratio as 50:1 (R=Me).

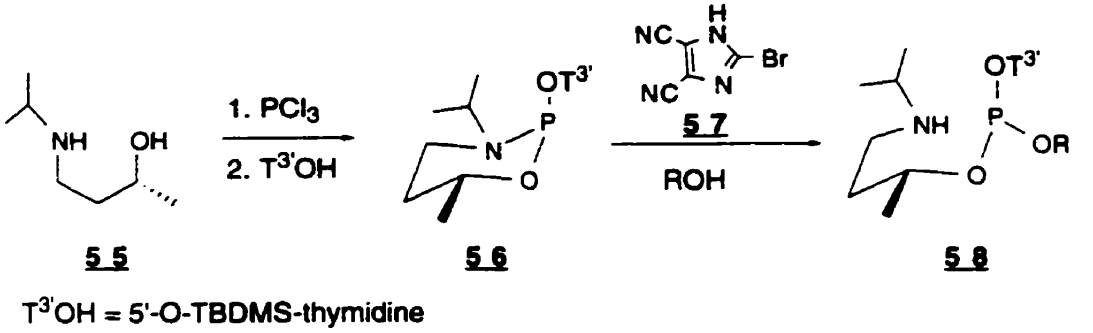

Figure 20. Chiral Cyclic Phosphoramidite Method by Xin and Just

Recently, new chiral auxiliaries **59**[137] and **60**[138,139] were synthesized. Both of them led to high ratio of dinucleoside phosphorothioate triesters **63** (97% de) and **64** (93% de). The chiral auxiliary on **63** can be removed by TFA, while **64** decomposes partially during deprotection with aqueous ammonia.

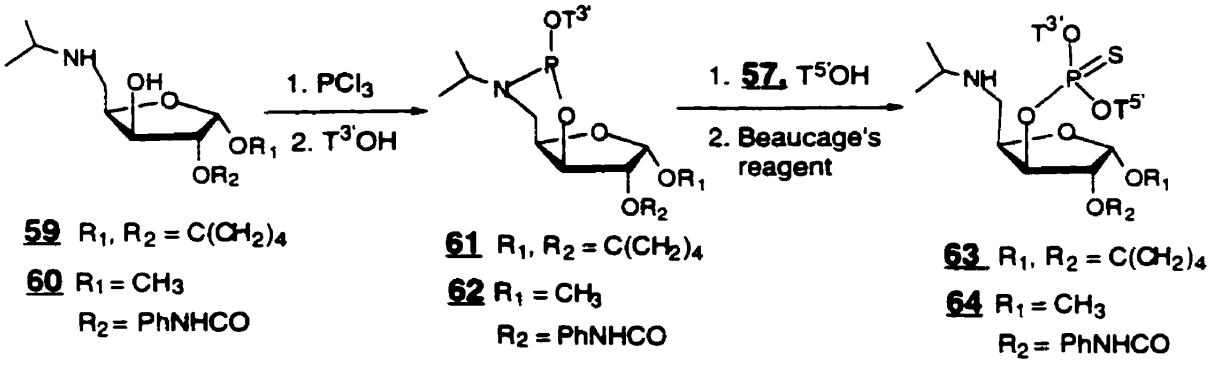

Figure 21. Improved Chiral Cyclic Phosphoramidite Method

However, this method is not adaptable to often acid-sensitive nucleosides because the catalyst **57** is too acidic and the reaction is too slow at -15 °C to be adapted to solid phase synthesis.

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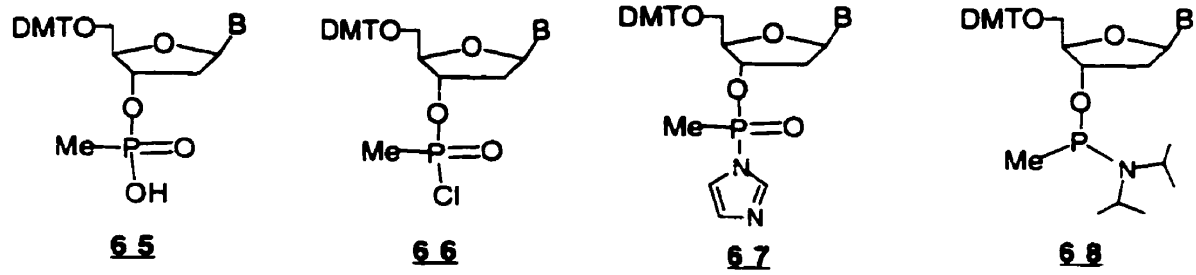
[137] Jin, Y.; Biancotto, G.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 973.
[138] Marsault, E.; Just, G. *Tetrahedron* **1997**, *53*, 16945.

1.5. Stereoselective Synthesis of Methylphosphonates

Oligonucleoside methylphosphonates can be prepared by many procedures. Methylphosphonic acid **65** and its derivatives **66** and **67** have been used as the synthetic intermediates. Acid **65** can be jointed with 3'-protected nucleoside to form methylphosphonates by using coupling reagents such as dicyclohexylcarbodiimide (DCC), mesitylenesulfonyl chloride, or mesitylenesulfonylnitrotriazole.[140] Methylphosphonic chloride **66** can react with 3'-protected nucleoside in the presence of pyridine, while **67** is activated by tetrazole.[141,142,143] The phosphorus atom in intermediates **65**, **66** and **67** is in the +5 oxidation state. Their coupling reactions are slow and give low yields. The more successful procedure for the synthesis of methylphosphonates makes use of methylphosphonamidite **68**.[144,145,146,147] As the phosphoramidite approach in the synthesis of oligonucleotide phosphate (Figure 12), the coupling reaction is carried out in the presence of tetrazole, followed by oxidation to the methylphosphonate linkage with aqueous iodine. However, all these methods yield a mixture of two diastereomers at the methylphosphonate linkage.



65 **66** **67** **68**

Figure 22. Synthons for Synthesis of Methylphosphonates

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[139] Marsault, E. Ph.D. Thesis, McGill University, 1996.
[140] Miller, P. S.; Agris, C. H.; Murakami, A.; Reddy, M. P.; Spitz, S. A.; Ts'o, P. O. P. *Nucleic Acids Res.* **1983**, *11*, 6225.
[141] Agarwal, K. L.; Riftina, F. *Nucleic Acids Res.* **1979**, *6*, 3009.
[142] Miller, P. S.; Agris, C. H.; Blandin, M.; Murakami, A.; Reddy, M. P.; Spitz, S. A.; Ts'o, P. O. P. *Nucleic Acids Res.* **1983**, *11*, 5189.
[143] Miller, P. S.; Reddy, M. P.; Murakami, A.; Blake, K. P.; Lin, S.-B.; Agris, C. H. *Biochemistry* **1986**, *25*, 5092.
[144] Sinha, N. D.; Groβbruchhaus, V.; Koster, H. *Tetrahedron Lett.* **1983**, *24*, 877.
[145] Dorman, M. A.; Noble, S. A.; McBride, M. J.; Caruthers, M. H. *Tetrahedron* **1984**, *49*, 95.
[146] Jager, A.; Engels, J. *Tetrahedron Lett.* **1984**, *25*, 1437.
[147] Agrawal, S.; Goodchild, J. *Tetrahedron Lett.* **1987**, *28*, 3539.

The methodologies for the stereoselective synthesis of methylphosphonates are still limited. The common approach is the nucleophilic displacement at tetracoordinated phosphorus centers **69**. It was reported that p-nitrophenyl[148,149,150,151] or methylselenyl group[152] on **69** could be replaced by 3'-protected nucleoside to give chiral phosphonates **70** in the presence of tBuMgCl or DBU. Cormier reported that 1,1,1,3,3,3-hexafluoro-2-propanoxyl group also could be replaced by a nucleoside in the presence of t-BuMgCl.[153]

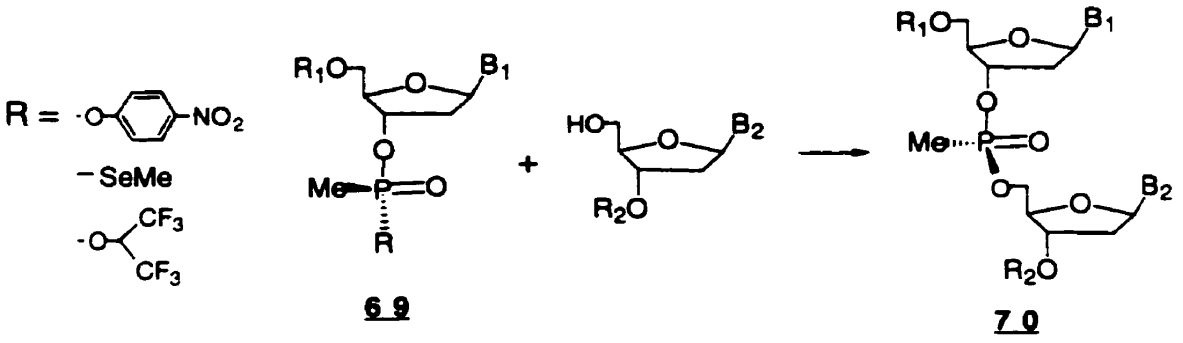

Figure 23. Synthesis of Chiral Methylphosphonates by Nucleophilic Displacement

Vaghefi et al. reported a tetracoordinated mixed anhydride of a nucleoside **71** which could be activated by $AgNO_3$ and coupled with methanol without epimerization.[154]

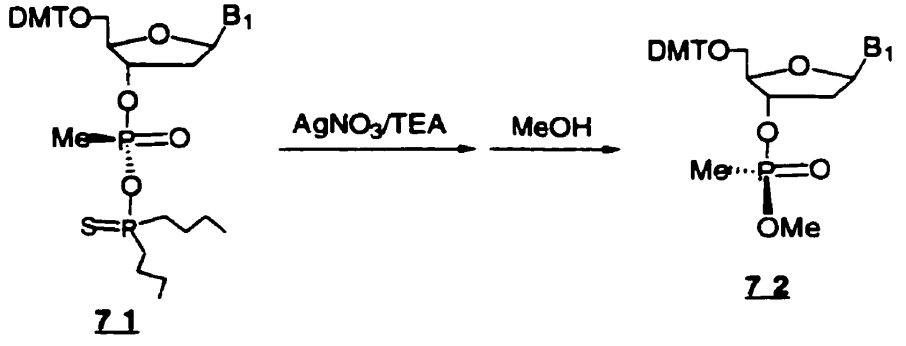

Figure 24. Mixed Anhydride Approach by Vaghefi et al.

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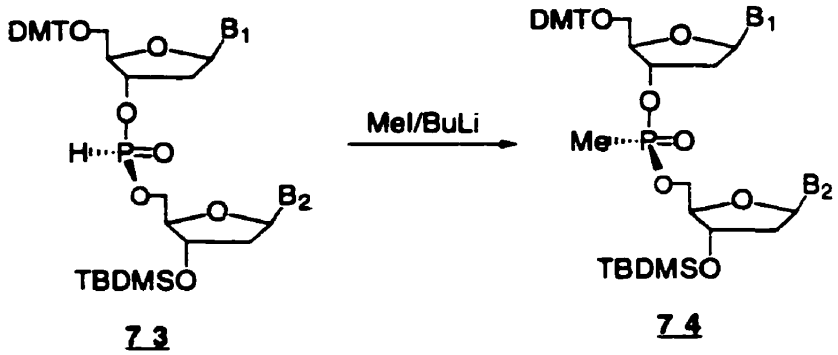
[148] Lesnikowski, Z. J.; Wolkanin, P. J.; Stec, W. J. *Tetrahedron. Lett.* **1987**, *28*, 5535.
[149] Lesnikowski, Z. J.; Jaworska, M.; Stec, W. J. *Nucleic Acids Res.* **1988**, *16*, 11675.
[150] Bec, C. L.; Wickstrom E. *Tetrahedron Lett.* **1994**, *35*, 9525-9528; *J. Org. Chem.* **1996**, *61*, 510.
[151] Jaworska-Maslanka, M. M.; Kacperczyk, W.; Korczynski, D.; Lesnikowski, Z. J. *Antisense & Nucleic Acid Drug Development* **1997**, *7(1)*, 23.
[152] Wozniak, L. A.; Pyzowski, J.; Wieczorek, M.; Stec, W. J. *J. Org. Chem.* **1994**, *59*, 5843.
[153] Cormier, J. F.; Pannunzio, T. *Tetrahedron Lett.* **1991**, *32*, 7161.
[154] Vaghefi, M. M.; Langley, K. A. *Tetrahedron Lett.* **1996**, *37*, 4853.

Seela et al. reported a synthesis of methylphosphonates by methylation of H-phosphonates. Only one dinucleoside methylphosphonate **74** was formed from a stereochemically pure H-phosphonate **73**.[124]



73 **74**

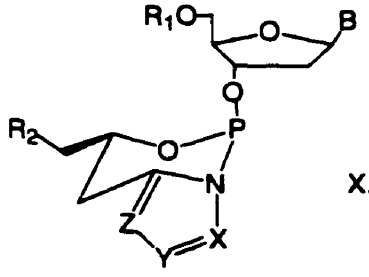
Figure 25. The Synthesis of Methylphosphonates *via* H-Phosphonates

However, in all above procedures, the chiral precursors **69**, **71**, **73** have to be separated chromatographically from their diastereomers.

Chapter II. The Stereoselective Synthesis of Phosphorothioates

2.1. Introduction

In the phosphoramidite approach for the synthesis of oligonucleotides, the use of tetrazole as a catalyst caused epimerization at the phosphorus center (Figure 13).[103] Our laboratory's cyclic phosphoramidite method demonstrated that diastereomerically pure cyclic phosphoramidites could be obtained by using γ-aminoalcohols as chiral auxiliaries, and some degree of stereocontrol was achieved by using dicyano bromoimidazole **57** as a catalyst in the coupling reaction.[135] However, due to its acidic nature, the catalyst **57** caused new problems, such as the deprotection of the trityl group which is used as a protecting group for nucleosides, and high stereoselectivity is achieved only at low temperature (< -15 ^{0}C).[137] Thus, the cyclic phosphoramidite procedure is not a general solution to this problem.

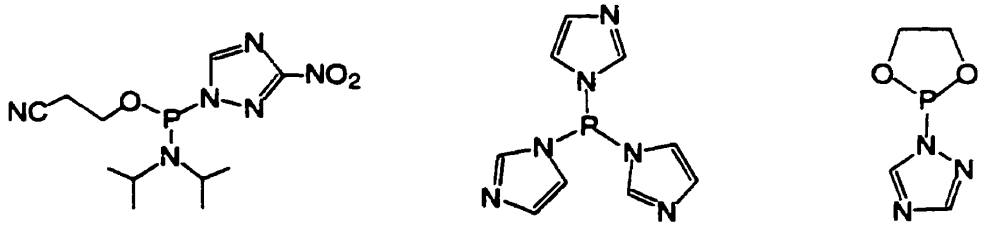


75

We tried to develop a procedure avoiding acidic catalysts. In doing so, we had to find a new leaving group to replace the amine moiety of phosphoramidite such as **56**, which could be substituted by a nucleoside without using any acidic catalysts. In the mechanism of epimerization caused by tetrazole (Figure 13), an intermediate phosphorotetrazolide **37** was formed, and there was a fast equilibration between its two isomers, which was the reason for epimerization. Since the tetrazole group on **37** could be

directly substituted by a nucleoside to form a phosphite, we proposed to replace the amine moiety with an azole group to form a cyclic oxazaphosphorine like **75**, in other words incorporating a catalyst into the precursor. This should eliminate the using of acidic catalysts in the coupling reaction with a nucleoside.

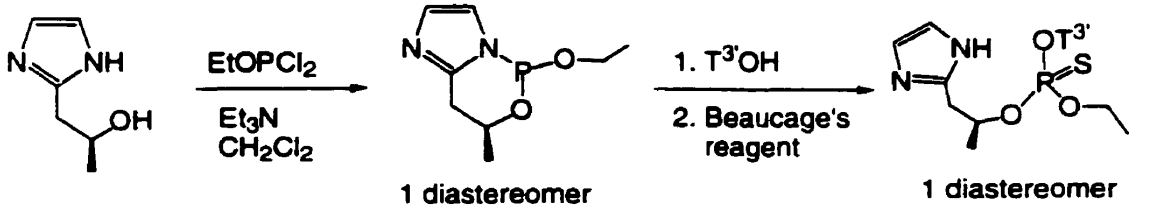
In literature, several azole substituents have already been used as leaving groups on trivalent phosphorus compounds. For example, the 3-nitro-1,2,4-triazole group on **76** can be easily replaced by a nucleoside to form phosphoramidite without using any acidic catalyst.[155] The imidazole groups in **77** can be quickly replaced by 1,2-diols to form cyclic dialkoxyphosphorimidazolides.[156] Phosphorotriazolide **78** was reported to be a good coupling reagent in the synthesis of polypeptides.[157]



76 **77** **78**

Figure 26. Trivalent Phosphorus Compounds Containing Azole Groups

In our lab, Dr. Marsault was the first person who tried to synthesize such a cyclic phosphoramidite analogue **75**.[158] He chose imidazole as the azole group and synthesized a chiral auxiliary **79**.



79 **80** **81**

Figure 27. The Imidazo-oxazaphosphorine Approach by Marsault and Just

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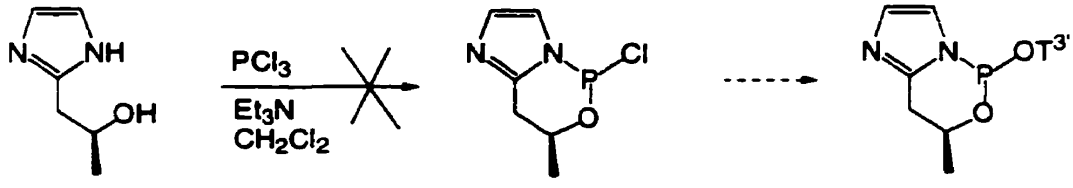
[155] Zhang, Z.; Tang, J. Y. *Tetrahedron Lett.* **1996**, *37*, 331.
[156] Shimidzu, T.; Yamana, K.; Kanda, N.; Kitgawa, S. *Bull. Chem. Soc. Jpn* **1983**, *56*, 3483.
[157] Kricheldorf, H. R.; Fehrle, M.; Kaschig, J. *Angew. Chem. Int. Ed. Engl.* **1976**, *15*, 305.
[158] Marsault, E.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 977.

From the reaction of **79** with dichloroethylphosphite, he obtained a diastereomerically pure imidazo-oxazaphosphorine **80**. As wished, the imidazole group of **80** was replaced by a nucleoside in the presence of triethylamine, and the reaction was stereospecific. Sulfurization of the reaction mixture with Beaucage's reagent afforded a diastereomerally pure phosphorothioate triester **81**. Dr. Marsault's work showed that the formation of a cyclic phosphoramidite analogue such as **75** was feasible.

However, a major problem for this approach is that the imidazo-oxazphosphorine **80** was too unstable to be handled routinely. The chiral auxiliary **79** could not be transformed to imidazole derivatives such as **82** or **83**, which are the precursors for the synthesis of chiral oligonucleotide phosphorothioates. Therefore the usefulness of imidazo-oxazaphosphorine of type **80** was limited.



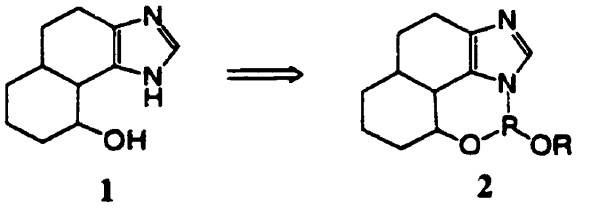
79 **82** **83**

Figure 28. Unable to Synthesize a Precursor **83** Containing a Nucleoside

In order to further develop this line of research, the first important thing was to find an azole group that could form a relatively stable compound with phosphorus. Then we considered using this group to develop a chiral auxiliary for the formation of a cyclic phosphoramidite analogue such as **75**, and investigating the reactivity and diastereoselectivity of the replacement reaction with a nucleoside. Since our final goal was to develop a method for the stereoselective synthesis of oligonucleotide phosphorothioates on solid phase, the chiral auxiliary developed should also be removable at the end.

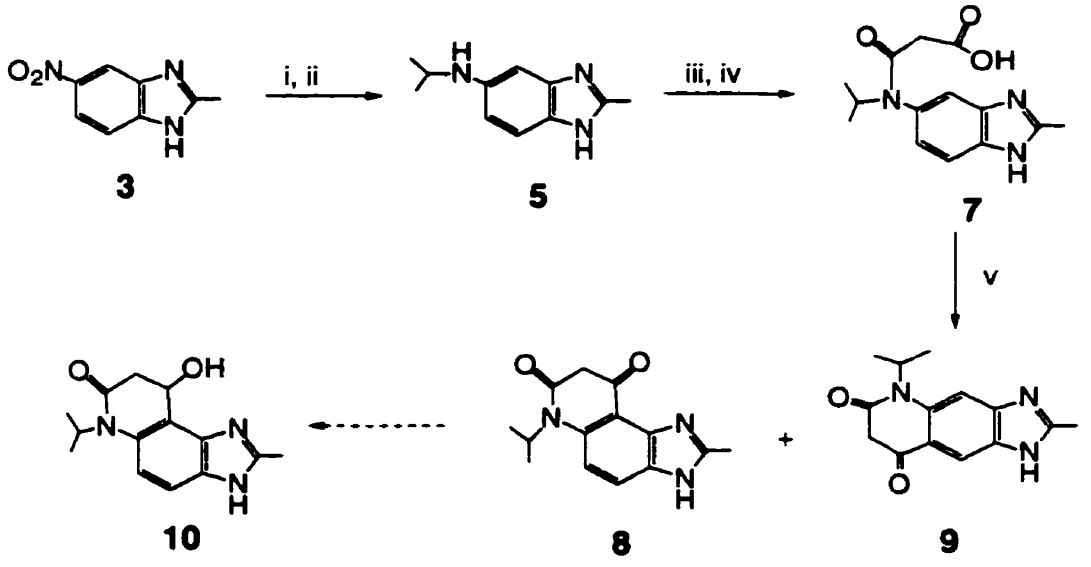
2.2. The Search for a Stable Phosphorazolide

Our initial idea for improving the stability of imidazo-oxazaphosphorine as **80** was to use a rigid imidazole auxiliary like **1**.[159] There will be less negative entropy changes in the formation of **2** from a rigid **1**, and this might improve the stability of imidazo-oxazaphosphorine **2**.



Scheme 1. Proposed Rigid Chiral Auxiliary

In order to synthesize a compound like **1**, 2-methyl-5-nitro-benzimidazole **3** was chosen as the starting material since it already has two rings.



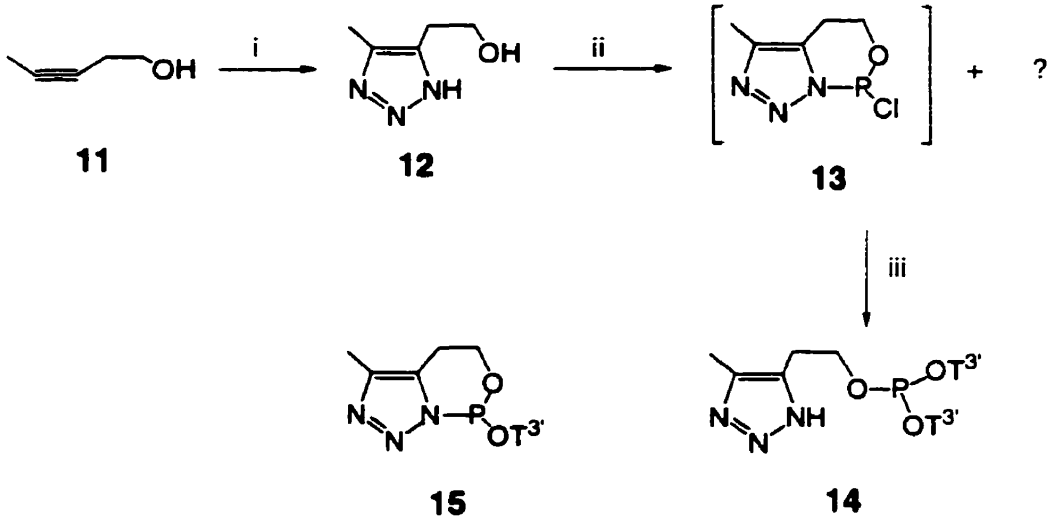
i). H_2, Pd/C, EtOH **4**. ii). acetone, $NaBH_3CN$, pH 6. iii). Methyl malonyl chloride, Et_3N, CH_2Cl_2, **6**. iv). LiOH, CH_3OH/H_2O (3:1). v). a. SO_2Cl_2, CH_2Cl_2. b. $AlCl_3$, $C_6H_5NO_2$.

Scheme 2. The Approach for the Synthesis of Rigid Auxiliary **10**

[159] For the convenience, compounds related to my research are renumbered, but without underline.

Catalytic hydrogenation[160] of the nitro group, followed by reductive alkylation[161] of the amine with $NaBH_3CN$ and acetone gave amine derivative **5** in 87% yield. Acylation of **5** with methyl malonyl chloride followed by hydrolysis of the methyl ester with LiOH afforded acid derivative **7** in 92% yield. However, the intramolecular Friedel-Crafts acylation of **7** gave a low yield of desired product **8** (<10%). It was reported that in benzimidazole, if the 5-substituent is powerfully electron-releasing, the second substituent enters at the 4-position.[162,163] In our case, with the amide group at C-5, we obtained both C-4 and C-6 acylation products **8** and **9** in almost equal amounts as established by ^{1}H-NMR. This procedure for the synthesis of a rigid imidazole auxiliary **10** was impractical. Many other even less successful attempts were made to synthesize this type of system. This approach was therefore discontinued.

Besides imidazole, the other azole group considered for our purpose was triazole since its use was also reported in the literature (Figure 26, 76, 78).[155,157] As a model reaction, a triazole derivative **12** was synthesized from alkyne **11** by [3+2] addition with azide.[164]



i). $TMSN_3$, 114 °C. ii). PCl_3, Et_3N. iii).5'-O-TBDMS-thymidine ($T^{3'}OH$).

Scheme 3. The Reaction of Triazole Auxiliary **12** with PCl_3 and Nucleosides

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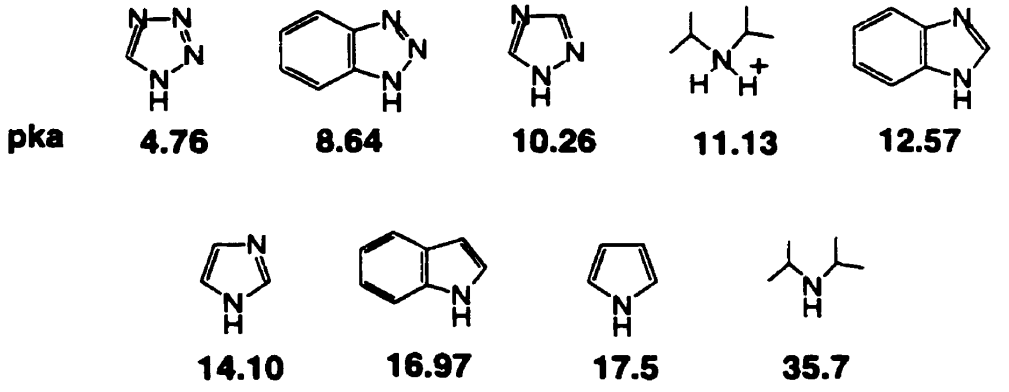
[160] Kirk, K. L.; Cohen, L. A. *J. Org. Chem.* **1969**, *34*, 384.
[161] Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
[162] Preston, P. N. Benzimidazoles and congeneric tricyclic compounds, Part 1, John Wiley & Sons, p83.
[163] Fries, K. *Justus Liebigs Ann. Chem.* **1927**, *454*, 121.
[164] Banert, K. *Chem. Ber.* **1989**, *122*, 911.

However, the reaction of triazole derivative **12** with PCl_3 did not afford a clean chloro derivative **13**. Several peaks were observed at the range of 80-180 ppm in the ^{31}P NMR spectrum, even though the reaction mixture was kept at 45 ^{0}C for more than 3 weeks. When 5'-O-TBDMS-thymidine ($T^{3'}OH$) was introduced to this reaction mixture, several peaks were observed around 141 ppm, which is the region characteristic for phosphite triesters. There was no evidence for the formation of desired triazole intermediate **15** having its ^{31}P NMR around 130 ppm. After column chromatography on silica gel, a phosphite triester **14** was obtained as the major product. This result, the formation of phosphite triester **14**, clearly demonstrated that triazole did not form a stable intermediate with phosphorus as illustrated in **13** and **15**. Both the triazole and chloro group were substituted by the nucleoside under the condition used.

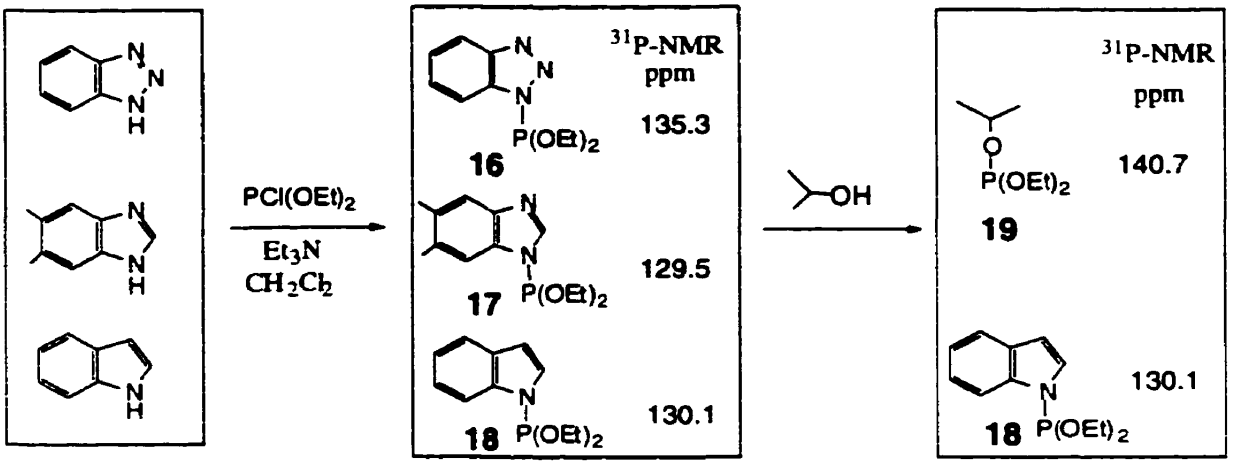
In order to get a stable precursor like **15**, the N-P bond's strength had to be increased. This led us to investigate other aromatic heterocycles. Literature pKa values are listed in Scheme 4, and indicate that indole and pyrrole are less acidic than imidazole and triazole. The bond of the indole or pyrrole with phosphorus should therefore be more stable than the one with the nitrogen of imidazole or triazole.



Scheme 4. The pKa of Aromatic Heterocycles and Diisopropylamine

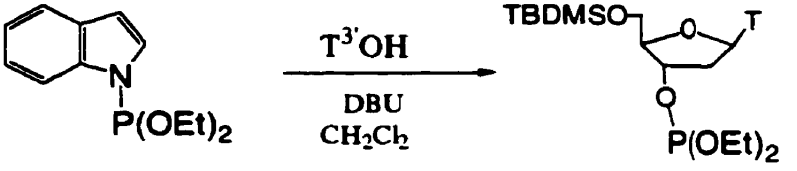
A set of model reactions were carried out to evaluate stabilities of these heterocyclic phosphorazolides. Aromatic heterocycles were first reacted with diethyl chlorophosphite in the presence of triethylamine. We found that benzotriazole, 5,6-dimethyl-benzimidazole, and indole all could form corresponding phosphorazolide intermediates **16**, **17**, and **18** as

established by ^{31}P NMR (Scheme 5). When isopropanol was introduced to the reaction mixture, the phosphorazolides **16** and **17** immediately turned to phosphite triester **19**, in which the benzotriazole and benzimidazole were replaced by isopropanol.



Scheme 5. The Model Reaction for Searching a Stable Phosphorazolide

To our great satisfaction, the indole derivative **18** was stable and no reaction took place when treated with isopropanol in the presence of triethylamine. It was stable enough to be purified by flash silica gel column chromatography. Therefore, indole met our first requirement for the formation of a stable phosphorazolide. The next thing of concern was the replacement of the indole group by a nucleoside. Since the imidazole group in trivalent phosphorus compounds such as **80** or **17**, could be directly replaced by an alcohol in the presence of the weak base triethylamine, we reasoned that the indole group of **18** might be replaced by a nucleoside in the presence of a stronger base. We tried the reaction of **18** with 5'-O-TBDMS-thymidine ($T^{3'}OH$) and 1,4-diazabicyclo[5,4,0]undec-7-ene (DBU).[165]



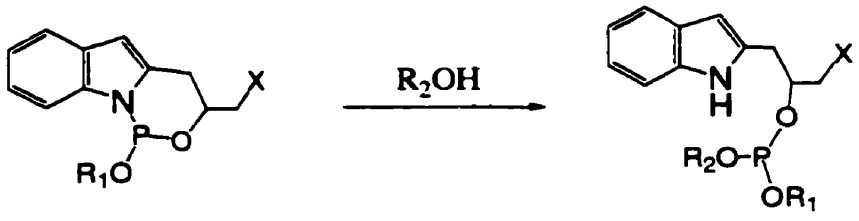
18 **20**

Scheme 6. The Displacement of Indole Group with a Nucleoside

[165] Lesnikowski, Z. J.; Zabawska, D.; Jaworska-Maslanka, M. M.; Schinazi, R.; Stec, W. J. *New J. Chem.* **1994**, *18*, 1197.

To our great excitement, the indole group of **18** was quantitatively substituted by the thymidine derivative $T^{3'}OH$ within several minutes to form a phosphite triester **20**.

Indole had all the properties for a good leaving group which we were looking for. Therefore, we thought that a indole-oxazaphosphorine like **21** could be a good precursor for the stereoselective synthesis of phosphorothioates, and we next focused on the synthesis of indole-oxazaphosphorine **21**, and the investigation of its reactivity and stereoselectivity in the reaction with a nucleoside.



21 **22**

Scheme 7. Indole-oxazaphosphorine

2.3. The Synthesis of Indole-oxazaphosphorine

The simplest auxiliary containing an indole and a chiral alcohol is 1-(indol-2-yl)-isopropanol **26**. We first synthesized racemic **26** to carry out model reactions. The synthesis of regiospecifically substituted indoles is well established.[166] 2-Substituted indole derivatives can be easily prepared by using N-phenylsulfonyl 2-lithioindoles.[167] Reaction of the lithioindole with propylene oxide gave alcohol derivative **25**, as shown in Scheme 8. Removal of the phenylsulfonyl group of **25** with potassium hydroxide afforded racemic 1-(indol-2-yl)-isopropanol **26** as a light amber oil.

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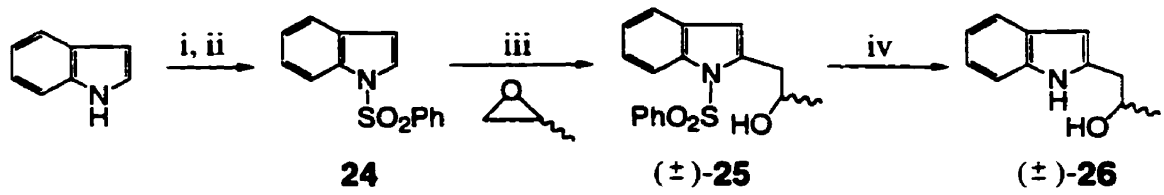
[166] Saulnier, M. G.; Gribble, G. *J. Org. Chem.* **1982**, *47*, 757.
[167] Hasan, I.; Marinelli, E. R.; Lin, L.-C. C.; Fowler, F. W.; Levy, A. B. *J. Org. Chem.* **1981**, *46*, 157.

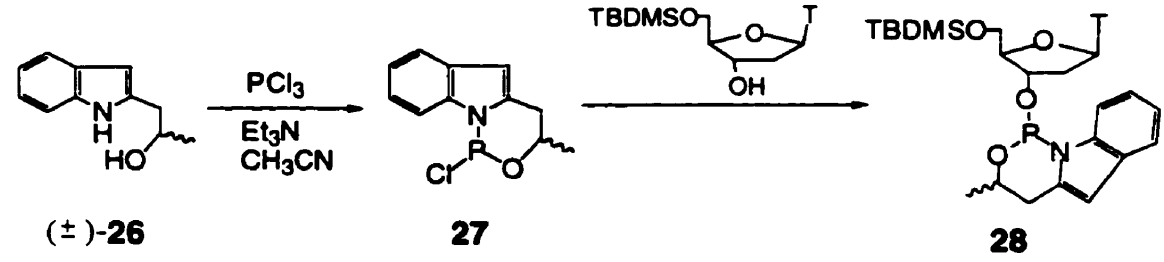


24 (±)-**25** (±)-**26**

i). n-BuLi, THF. -78 °C. ii). $PhSO_2Cl$, 90.6%. iii). n-BuLi, THF. -78 °C, 72.8%. iv). KOH, CH_3OH/H_2O (3:1), 98%.

Scheme 8. The Synthesis of Racemic 1-(Indol-2-yl)-isopropanol **26**

Alcohol (±)-**26** was then reacted with PCl_3 in the presence of triethylamine. The reaction was initially run in a dry NMR tube and followed by ^{31}P NMR. Equimolar acetonitrile solutions of (±)-**26** and PCl_3 were allowed to react at 0 °C under argon. After a few minutes, the total disappearance of the peak corresponding to PCl_3 at 221 ppm was observed, and several peaks appeared around 140 - 150 ppm. With time, one peak grew while the others diminished, and finally only one big peak was observed at 144 ppm, corresponding to the formation of chloro derivative **27** (Scheme 9). In acetonitrile, this transformation took one day at 60 °C, whilst in dichloromethane , it took a week at 40 °C. When only one peak remained at 144 ppm, one eq. of 5'-O-TBDMS-thymidine ($T^{3'}OH$) was added. Within a few minutes, ^{31}P NMR showed a complete consumption of the intermediate **27**, and new peaks were observed around 121 ppm, corresponding to the formation of indole-oxazaphosphorines **28**.



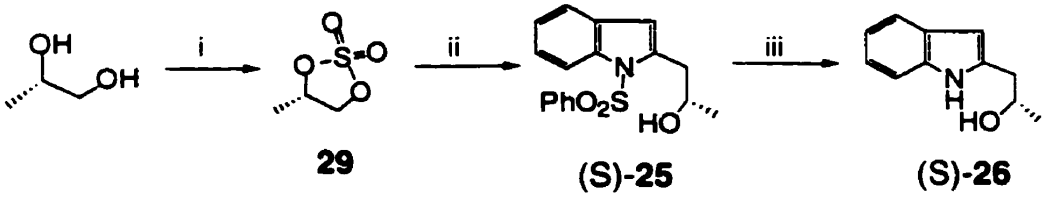
(±)-**26** **27** **28**

Scheme 9. The Synthesis of Indole-oxazaphosphorine **28** with Racemic **26**

Indole-oxazaphosphorine **28** was purified by flash silica gel column chromatography and characterized by NMR. In its ^{31}P NMR, there were only two major peaks at 120.7 ppm and 120.5 ppm. This indicated that the formation of indole-

oxazaphosphorine **28** was stereospecific, otherwise four isomers in the same ratio would be obtained from racemic auxiliary **26**. The results from this model reaction clearly demonstrated that the indole-oxazaphosphorine approach was feasible.

As a next step, chiral auxiliary (S)-**26** was synthesized. In the procedure of Scheme 8, we could have obtained chiral (S)-**26** if (S)-propylene oxide had been used as the electrophile. However, the chiral (S)-propylene oxide is very expensive, and we decided to use the less expensive (S)-propanediol as the chiral starting material. (S)-propanediol was first transformed to a reactive electrophilic cyclic sulfate **29**.[168,169] Using the same procedure as in the formation of racemic **25**, cyclic sulfate **29** was treated with the anion of **24**. Removal of the sulfate group with 20% H_2SO_4, and of the phenylsulfonyl group with KOH afforded the chiral (S)-**26**, as outlined in Scheme 10.



29 (S)-**25** (S)-**26**

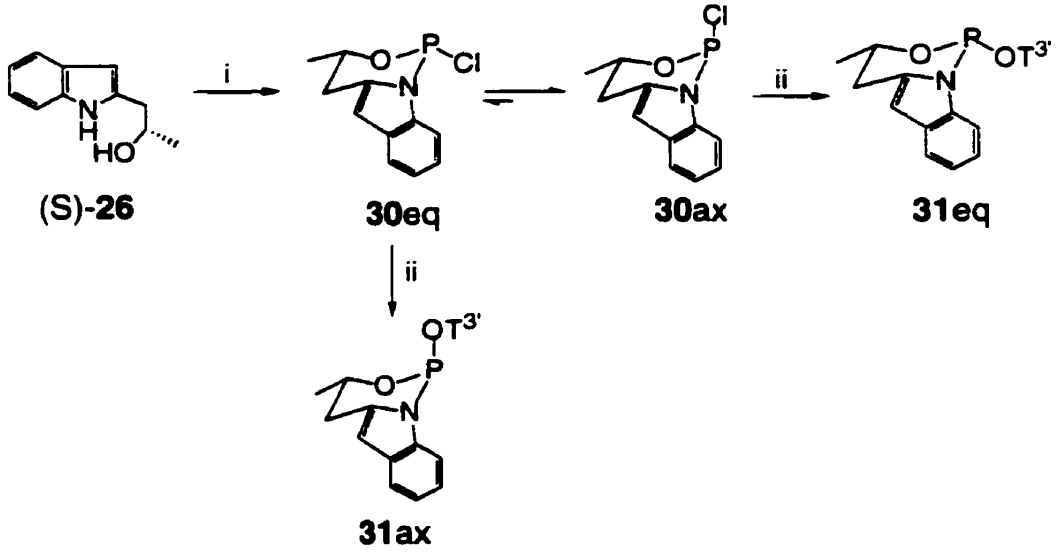
i) a. $SOCl_2$, CCl_4, 60 °C. b. $NaIO_4$, $RuCl_3 3H_2O$, CH_3CN/H_2O, 25 °C, 98%. ii) 1-phenylsulfonyl-indole **24**, n-BuLi, -78 °C - 25 °C, overnight, then add 20% H_2SO_4 and stir for 3 hours, 87%. iii) KOH, CH_3OH/H_2O (3:1), reflux, 99%.

Scheme 10. The Synthesis of Chiral (S)-**26**

The reaction of (S)-**26** with PCl_3 was carried out as for (±)-**26.** Equimolar acetonitrile solutions of (S)-**26** and PCl_3 were allowed to react at 0 °C under argon for half an hour, then the mixture was warmed up to 60 °C. The warming was continued (about 10 hours) until the ^{31}P NMR showed a major peak at 144 ppm, which indicated the formation of phosphorochloridite **30** (Scheme 11). It probably exists as a rapidly equilibrating mixture of **30**ax and **30**eq, in which **30**ax predominates (*vide infra*). The mixture was cooled to 0 °C again, and a solution of 5'-O-TBDMS-thymidine ($T^{3'}OH$) in CH_2Cl_2 was added. In the ^{31}P NMR of the reaction mixture, a new set of peaks was observed around

[168] Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.
[169] Kim, B. M.; Sharpless, K. B. *Tetrahedron Lett.* **1989**, *30*, 655.

120 ppm while the one at 144 ppm disappeared. After half an hour, the crude mixture was purified by silica gel chromatography to remove unreacted thymidine and triethylammonium chloride to give indole-oxazaphosphorine **31**. There were two peaks in its ^{31}P NMR, a major one at 120.74 ppm, and a minor one at 121.56 ppm in a ratio of 9:1, corresponding to the formation of two diastereomer **31eq** and **31ax**, as shown in Scheme 14A. These two diastereomers could not be separated by flash chromatography on silica gel.



i) PCl_3, CH_3CN/Et_3N, 0 °C - 60 °C. ii) 5'-O-TBDMS-thymidine ($T^{3'}OH$).

Scheme 11. The Synthesis of Indole-oxazaphosphorine with (S)-**26**

In our laboratory's previous studies,[135,158] the two diastereoisomers at phosphorus of phosphoramidites **56** and imidazo-oxazaphosphorine **80** could be equilibrated by heating their solutions containing triethylammonium chloride to form a pure diastereomer, by virtue of the preferred axial orientation dictated by the anomeric effect. In marked contrast, **31ax** and **31eq** could not be equilibrated by heating in the presence of either acid (silica gel, $R_3N{\cdot}HCl$) or base (triethylamine). Since **30ax** predominated in the formation of phosphorochloridite **30**, the equatorial compound **31eq** became the major one in indole-oxazaphosphorine **31** after the chloro group of **30** was attacked by thymidine in an S_N2 manner.

The ratio of two diastereoisomers of indole-oxazaphosphorine **31** was affected by the temperature at which $T^{3'}OH$ was added. At 20 °C, the ratio was 7:1; at lower temperature (0 – -78 °C), the ratio increased to 9:1, as shown in Table 2.

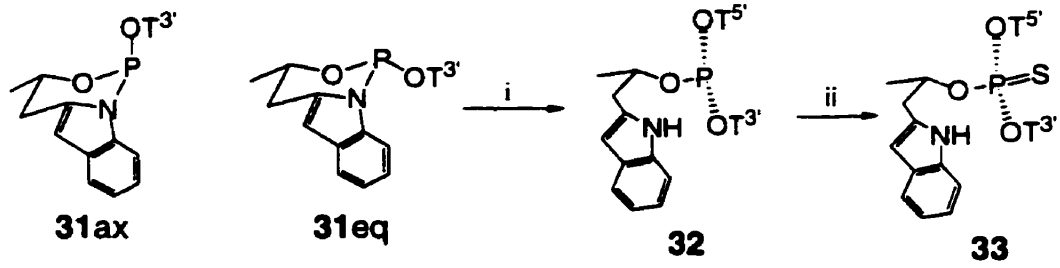
Table 2. The Temperature Effects on the Ratio of Two Diastereoisomers **31***

Temperature	60°C	25°C	0°C	-15°C	-78°C
Ratio of **31eq** : **31ax** 120.74 ppm : 121.56 ppm	5.4:1	7.2 : 1	9.3 : 1	9.2 : 1	9.0 : 1

*The crude mixture was passed through a short silica gel column to filter out triethylammonium chloride and eluted with dry CH_3CN. The solvent was evaporated, and the resulting sample was dried under vacuum for an hour. The sample was dissolved in $CDCl_3$ and ready for ^{31}P NMR determination.

2.4. The Displacement of the Indole Group in Indole-oxazaphosphorine with a Nucleoside

We then attempted to open the ring of indole-oxazaphosphorine **31** with a nucleoside. The reaction of **31** with 3'-O-TBDPS-thymidine ($T^{5'}OH$) was done in the presence of DBU.

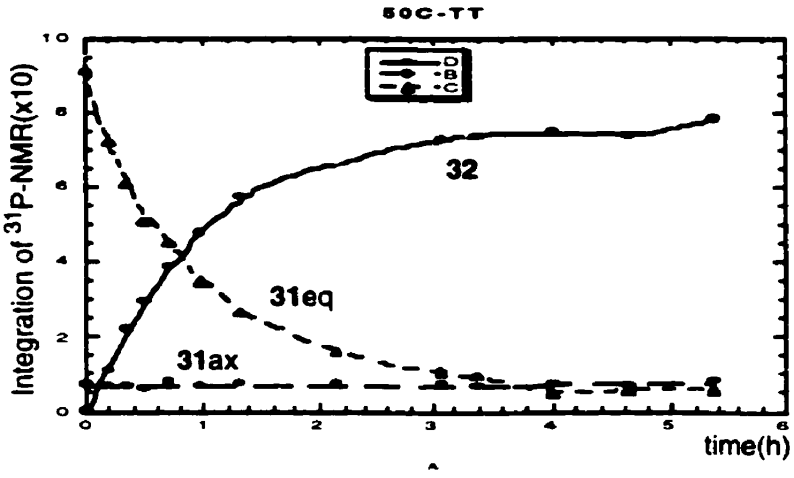


i) 3'-O-TBDPS-thymidine ($T^{5'}OH$), DBU, **32**. ii) Beaucage's reagent.

Scheme 12. The Synthesis of Phosphorothioate Triester **33**

Since the equatorial cyclic phosphorus compound is thermodynamically less stable, the major **31eq** reacted faster with 3'-O-TBDPS-thymidine than the minor **31ax**. Excitingly, the ratio of two diastereomers of phosphite triester **32** was improved during this coupling reaction.

In order to investigate the reactivities of **31ax** and **31eq**, we followed a reaction which was run in a dry NMR tube by ^{31}P NMR. One eq. of indole-oxazaphosphorine **31** was reacted with 1 eq. of thymidine $T^{5'}OH$ in the presence of 1 eq. of DBU at 50 ^{0}C. Scheme **13** shows the ^{31}P NMR integration of reagent **31ax**, **31eq** and product **32** with time during the reaction. The integration of their ^{31}P NMR were compared with the one of trimethylphosphate which was added in the NMR tube as an internal standard. We can see that the peak for **31ax** almost did not change during the reaction while the one for **31eq** decreased constantly. After five hours, 95% of **31eq** was converted to phosphite triester **32** with a 142 ppm peak in ^{31}P NMR (Scheme 14**B**).

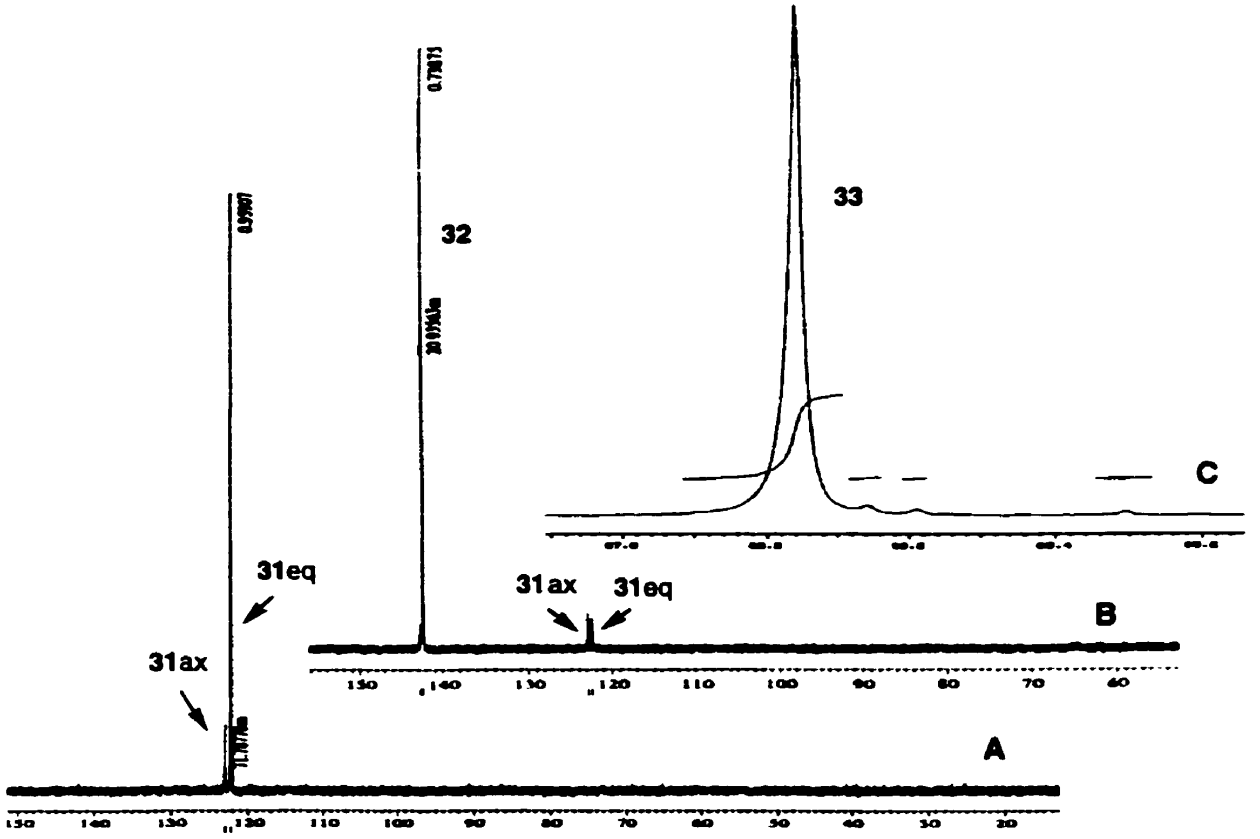


B. The integration of indole-oxazaphosphorine **31ax**. **C.** The integration of indole-oxazaphosphorine **31eq**. **D.** The integration of phosphite triester **32**.

Scheme 13. The Coupling Reaction of **31** with $T^{5'}OH$ Followed by ^{31}P NMR

The next step was to sulfurize the phosphite triester **32.** When the reaction mixture containing DBU was directly treated with Beaucage's reagent, a certain amount of side product was observed with ^{31}P NMR at -2 ppm, which was not characterized. After filtration through a short silica gel column to remove DBU, triester **32** was treated with

Beaucage's reagent to give phosphorothioates **33.** In its ^{31}P NMR, there were two small peaks at 66.65 ppm and 66.59 ppm (in same intensity) near the major one 66.75 ppm, as shown in Scheme 14C. We tentatively chose the one at 66.59 ppm as the isomer of **33** since most phosphorus diastereomers have more than 0.1 ppm difference in their ^{31}P NMR. The ratio of the two diastereomers of phosphorothioates **33** was 73:1, in which the major isomer most probably has the Sp configuration.



A. The ^{31}P NMR of indole-oxazaphosphorine **31** ($CDCl_3$, 68.7 MHz). **B**. The ^{31}P NMR of the reaction of **31**(1 eq.) with $T^{5'}OH$ (1 eq.) in DBU (1 eq.) after 5 hours at 50 ^{0}C ($CDCl_3$, 68.7 MHz). **C**. The ^{31}P NMR of phosphorothioate triester 33 ($CDCl_3$, 125.7 MHz). The two small peaks at 66.65 ppm and 66.59 ppm (in same intensity) were not assigned.

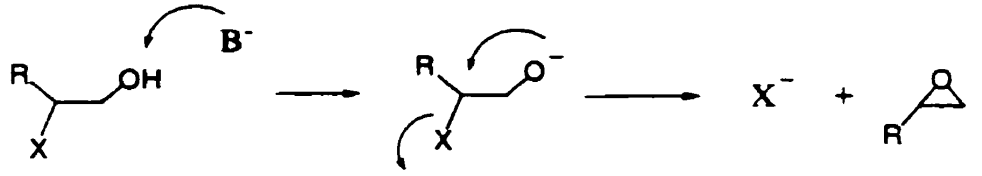
Scheme 14. The ^{31}P NMR of **31, 32, 33**.

The chiral auxiliary on **33** could not be removed with 28% ammonium hydroxide. Therefore, our next step was to develop chiral auxiliaries which could be removed at the phosphorothioate triester stage.

2.5. The Synthesis of Removable Chiral Auxiliaries

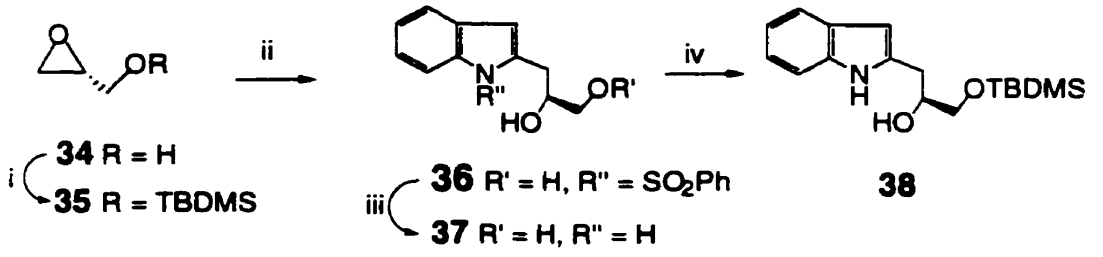
2.5.1. A Chiral Auxiliary with A Protected Hydroxyl Group

An internal S_N2 reaction is frequently used to prepare epoxides from β–halo alcohols,[170,171] as shown in Scheme 15. Therefore, a chiral auxiliary containing a protected hydroxyl group was considered.



Scheme 15. An Internal S_N2 for Releasing a X Group

We chose an indole derivative **38** containing a silyloxy group which could generate an alkoxide oxygen upon treatment of tetrabutylammonium fluoride (TBAF). (R)-glycidol **34** was used as the chiral starting material. The protected glycidol **35**[172] was reacted with the anion of **24** to give an alcohol **36**. Removal of the protecting groups with KOH afforded diol **37**. Then the primary hydroxyl group was selectively silylated to form chiral auxiliary **38**.



i) TBDMSCl, NEt_3, DMAP, CH_2Cl_2, 81.2%. ii) **24**, n-BuLi, -78 °C - 25 °C, overnight, 48.4%. iii). KOH, CH_3OH/H_2O (3:1), reflux, 87%. i) TBDMSCl, NEt_3, CH_2Cl_2, 84%.

Scheme 16. The Synthesis of a Protected Hydroxyl Auxiliary **38**

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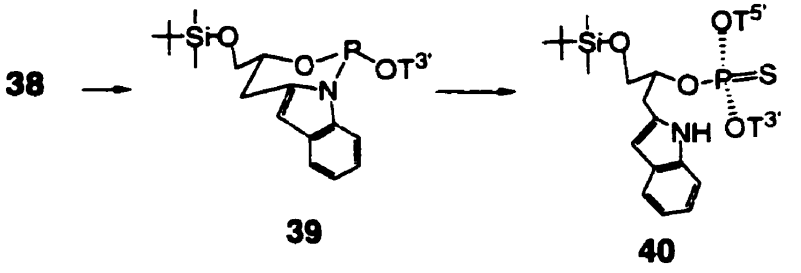
[170] Swain, C. G.; Ketley, A. D.; Bader, R. F. W. *J. Am. Chem. Soc.* **1959**, *81*, 2353.
[171] Knipe, G *J. Chem. Soc., Perkin Trans. 2* **1973**, 589.
[172] Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, *2*, 99.

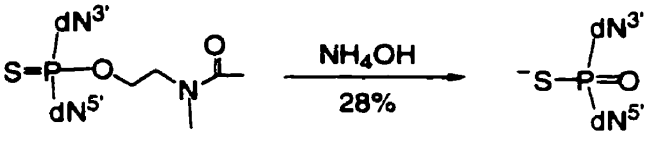
As in the formation of **33**, chiral auxiliary **38** was transformed to phosphorothioate triester **40** *via* indole-oxazaphosphorine **39**. However, the chiral auxiliary of **40** was not easily removed. Using TBAF, after a week at 50 ^{0}C, triester **40** was only partially released to phosphorothioate as established by ^{31}P NMR, as only a small peak at 54 ppm was observed. Thus, the silyloxy compound **38** is not a good choice for a removable auxiliary.



Scheme 17. The Phosphorus Compounds With Auxiliary **38**

2.5.2. A Chiral Auxiliary with an Acetamido Group

Iyer et al. found that reaction of a 2-acetamidoethylphosphate triester with aqueous ammonia gave the corresponding diester,[173,174] as shown in Scheme 18.



Scheme 18. The Neighboring Participation of Acetamidoethyl Group

We therefore decided to synthesize a chiral auxiliary **43** which incorporated an acetamido group. The synthesis of **43** was carried out starting from the diol **37** which was synthesized from glycidol **34** (Scheme 16). First the primary alcohol of **37** was selectively tosylated to form a sulfonate compound **41**, which then reacted with isopropylamine to give amine **42**. Acetylation of **42** afforded the desired acetamido derivative **43**.

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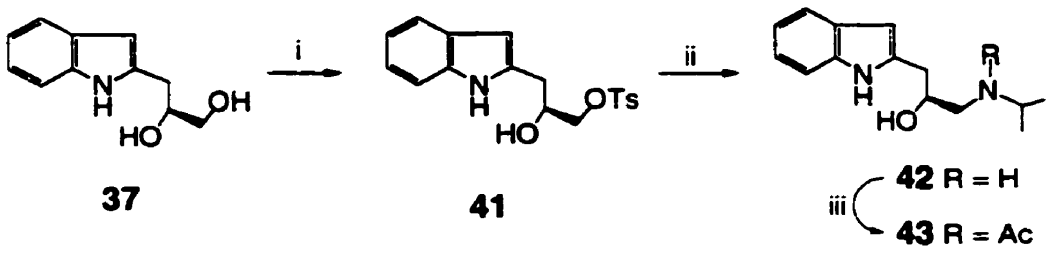
[173] Iyer, R. P.; Yu, D.; Devlin, T.; Ho, N.-H.; Agrawal, S. *J. Org. Chem.* **1995**, *60*, 5388.
[174] Iyer, R. P.; Yu, D.; Ho, N.-H.; Tan, W.; Agrawal, S. *Tetrahedron: Asymmetry* **1995**, *6*, 1051.

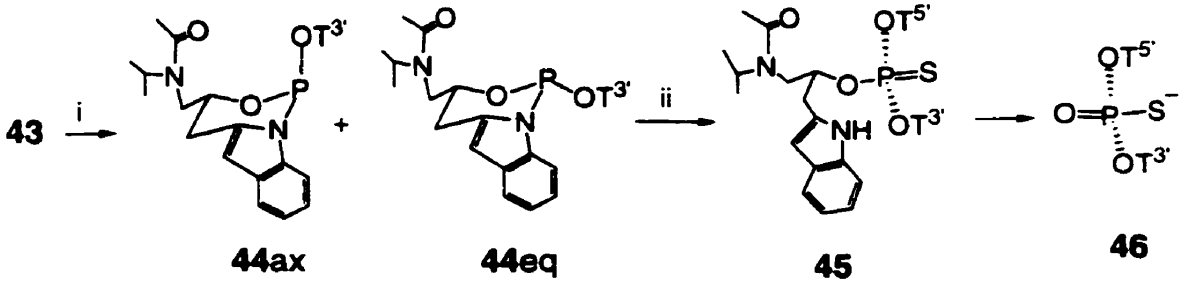


37 41

i) TsCl (1.1 eq.), pyridine, 0 °C, overnight, 99%. ii). Isopropylamine, 110 °C, overnight, 81%. iii) Acetic anhydride, CH_2Cl_2, 5 hours, then washed with saturated $NaHCO_3$ solution, 92%.

Scheme 19. The Synthesis of an Acetamido Auxiliary **43**

The reaction of acetamido derivative **43** with PCl_3 and $T^{3'}OH$ afforded indole-oxazaphosphorine **44eq** and **44ax** in a ratio of 6:1. It was transformed to phosphorothioate triester **45** (^{31}P NMR in THF, 68 ppm) as described for the corresponding indole derivative **33**.

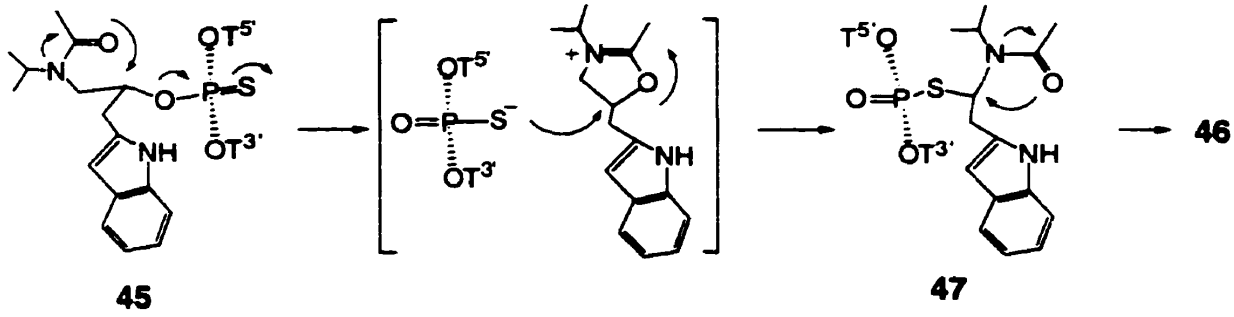


44ax 44eq 45 46

i). a. PCl_3, Et_3N, THF. b. 5'-O-TBDMS-thymidine ($T^{3'}OH$). ii). a. 3'-O-TBDPS-thymidine ($T^{5'}OH$), DBU. b. Beaucage's reagent.

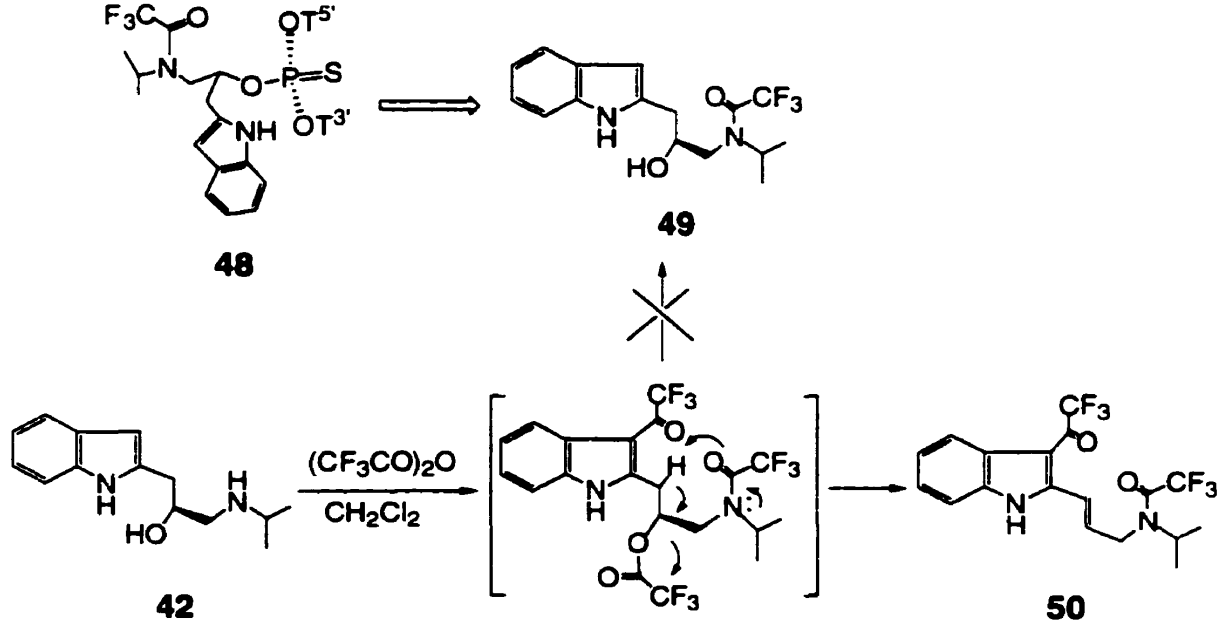
Scheme 20. The Synthesis of Phosphorothioate from **43**

Phosphorothioate triester **45** hydrolyzed spontaneously upon sulfurization in tetrahydrofuran solution within several hours to provide dithymidine phosphorothioate **46** as established by ^{31}P NMR (58 ppm in THF). By adding a base such as triethylamine, the reaction was complete in several minutes. During this transformation, an intermediate was observed at 22 ppm in the ^{31}P NMR spectrum, which is possibly a rearrangement intermediate **47** (Scheme 21).



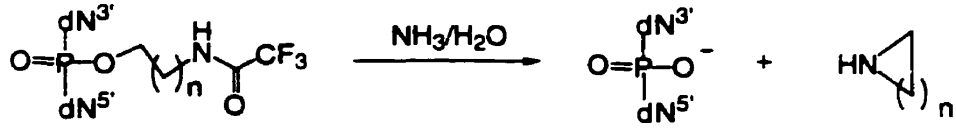
Scheme 21. A Possible Mechanism for the Formation of Intermediate **47**

This result demonstrated that the oxygen of amide group is too strong a nucleophile as it releases the phosphorothioate moiety spontaneously. Since the oxygen of trifluoroacetic amide is probably a less strong nucleophile, we tried to prepare N-trifluoroacetyl derivative **49** by the acylation of the amine derivative **42** with trifluoroacetic anhydride. However, the reaction gave olefin **50** as the only product. Unexpectedly, the 3-position of the indole was also acylated under the conditions used. One explanation for the formation of **50** might be that the amide group helped in the β-elimination, as shown in Scheme 22. On the other hand, the acidity of the α-proton at 2-position of the indole is increased by the 3-position's trifluoroacetyl group and makes this β-elimination possible.



Scheme 22. The Acylation of **42** with Trifluoroacetic Anhydride

Recently, Beaucage and co-workers reported that trifluoroacetamidobutyl or trifluoro-acetamidopentyl could serve as protecting groups (Scheme 23), and deprotection was achieved with aqueous ammonia.[175]



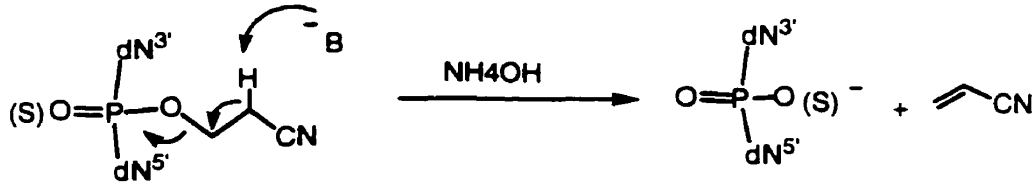
51
n = 3, 4

52 **53**

Scheme 23. Trifluoroacetic Amide Derivatives by Beaucage et al.

2.5.3. A Chiral Auxiliary with a Cyano Group

The commonly used deprotection in normal DNA synthesis is an ammonium hydroxide mediated β-elimination of a cyanoethylphosphate triester, as shown in Scheme 24. The acidity of the proton at the α position to the cyano group and the leaving ability of the phosphate group make this β-elimination possible with a weak base such as ammonium hydroxide in a short time.

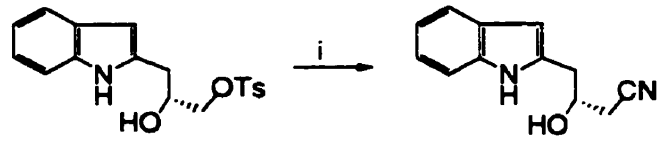


Scheme 24. β-Elimination of a Cyanoethylphosphate Triester

Therefore using cyano derivative **54** as a chiral auxiliary was considered. Cyano derivative **54** was prepared by the reaction of tosylated derivative **41** with cyanide salts in DMF. As shown in Scheme 16 and Scheme 19, tosylated **41** was synthesized from glycidol **34**. From commercial available (S)-glycidol (98% ee, Aldrich Chemical Co.), we obtained cyano derivative (R)-**54** but only in 90% ee as was analyzed by HPLC with a chiral column. We have not yet been able to elucidate the reason for this partial

[175] Wilk, A.; Srinivasachar, K.; Beaucage, S. L. *J. Org. Chem.* **1997**, *62*, 6712.

epimerization. The chirality of **54** was improved by recrystallization from $CHCl_3$. After recrystallization, we obtained (R)-**54** in 96% ee from the recrystallization mother liquor.



(R)-**41** (R)-**54**

i) NaCN/ LiCN, DMF, 100 °C, 1 hour,

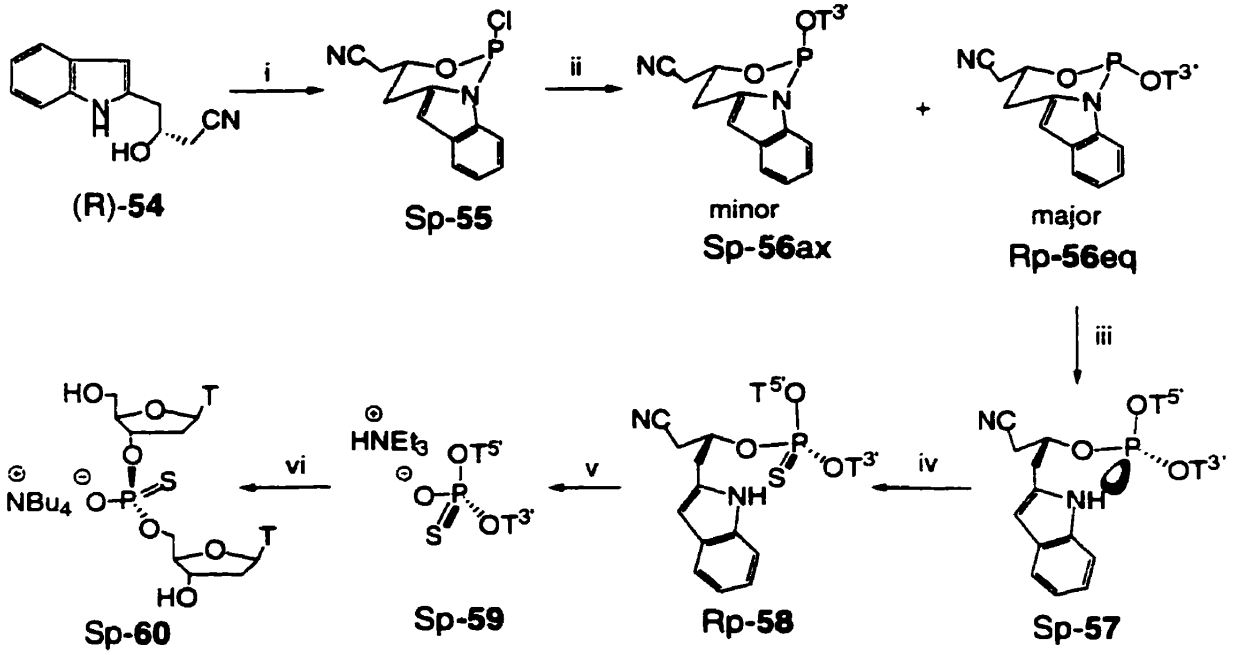
Scheme 25. The Synthesis of Chiral Auxiliary **54**

Interestingly, in THF at -78 °C, the reaction of (R)-**54** with PCl_3 was complete in several minutes to give phosphorochloridite **55** with a major peak at 144 ppm. $T^{3'}OH$ was then added at -78 °C. After half an hour, the reaction mixture was purified by column chromatography to provide a mixture of a major **56**eq and a minor **56**ax stereoisomer in a ratio of 30:1 as established by ^{31}P NMR, 120.78 ppm (major), 120.65 ppm (minor). Although cyano (R)-**54** only has 96% ee, only two diastereomers of indole-oxazaphosphorine **56** were observed.

As indole oxazaphosphorine **31**, equatorial indole-oxazaphosphorine **56**eq reacted faster than the axial one **56**ax. 1.3 eq. of **56** was treated with 1 eq. of $T^{5'}OH$ to afford phosphite triester Sp-**57**. With 2 eq. or 5eq of DBU at room temperature, the coupling was complete in less than 10 or 5 minutes, respectively. Interestingly, the corresponding reaction of **31** carrying no cyano group with $T^{5'}OH$ required several hours. Phosphite triester Sp-**57** was stable to DBU under the conditions used. After filtration through a short silica gel column to remove DBU, sulfurization with Beaucage's reagent afforded phosphorothioate triester Rp-**58** with a peak at 66.55 ppm in ^{31}P NMR spectrum.

The chiral auxiliary on **58** was easily removed with 28% ammonium hydroxide at 50 °C for 30 minutes to form Sp-**59** (^{31}P NMR, 58.96 ppm). Deprotection of silyl groups with TBAF afforded dithymidine phosphorothioate Sp-**60**. We found that the two diastereomers of Sp- and Rp-**60** can be easily identified by the ^{31}P NMR when D_2O is used as a solvent. There is more than 0.3 ppm difference in their chemical shift (Sp-**60**, 55.55 ppm; Rp-**60**, 55.87 ppm) and the Rp-isomer is at lower field, while in CD_3OD, the peaks of the two isomers are very near (less than 0.1 ppm difference, Sp-**60**, 59.07 ppm; Rp-**60**,

58.99 ppm) and the Sp-isomer is at lower field. Only one diastereomer Sp-**60** (^{31}P NMR in D_2O, 55.50 ppm) was identified from experiment.



i) PCl_3, THF, Et_3N (3.3 eq.), 0 °C, ii) $T^{3'}OH$, 0 °C iii) $T^{5'}OH$, DBU. iv). Beaucage's reagent. v) 28% NH_4OH, 55 °C 0.5 hour, flashed chromatography on silica gel (acetone:triethylamine 10:1). vi) 1 M TBAF in DMF.

Scheme 26. The Stereoselective Synthesis of Phosphorothioate with **54**

In a parallel run, from (R)-glycidol, cyano (S)-**54** was obtained and its chirality was also improved to 96% ee after recystallization. In THF at 0 °C, the reaction of cyano (S)-**54** with PCl_3 and $T^{3'}OH$ provided indole-oxazaphosphorine **56** with two diastereomers, ^{31}P NMR, 120.72 ppm (minor), 120.54 ppm (major) in a ratio of 1:6. The ratio of two diastereomers **56** was affected by the temperature and the work-up conditions. Since the major equatorial **56** is more reactive, it is easily hydrolyzed during the work-up such as during silica gel chromatography.

One equivalent of **56** was treated with 1 eq. of $T^{5'}OH$ in the presence of 2 eq. of DBU. After 5 minutes, DBU was filtered off and sulfurization with Beaucage's reagent provided phosphorothioate triester Sp-**58** (^{31}P NMR in $CDCl_3$, 66.31 ppm). It was difficult to identify the other isomer from its ^{31}P NMR. Cleavage of the chiral auxiliary

with aqueous ammonia afforded phosphorothioate diester Rp-**59** (^{31}P NMR in CD_3OD, 59.08 ppm). Removal of silyl groups with TBAF gave dimer **60**, and two diastereomers were identified from their ^{31}P NMR (CD_3OD), major Rp-**60** (58.93 ppm) and minor Sp-60 (58.99 ppm) in a ratio of 21:1. These two isomers were confirmed by their ^{31}P NMR in D_2O.

The absolute stereochemistry of dimers Rp-**60** and Sp-**60** were confirmed by snake venom phosphodiesterase and P1 nuclease digestion and HPLC analysis.[176]

At this stage, we demonstrated that indole-oxazaphosphorine **56** is a useful intermediate for the stereoselective synthesis of chiral phosphorothioate in solution phase, and the cyano chiral auxiliary could be easily removed at the end by aqueous ammonia. Our next step was to develop this procedure on solid support.

2.6. The Studies on Solid Support

In our procedure (Scheme 26), the coupling reaction of indole-oxazaphosphorine **56** with a nucleoside was done in the presence of DBU. It was reported that DBU partially cleaves the standard linker (-$COCH_2CH_2CO$-LCA-) and releases the nucleoside from the solid support.[177,178] Therefore, 5'-O-DMT-thymidine was immobilized on controlled pore glass (CPG) *via* a DBU-resistant sarcosinyl-succinoyl linker ($COCH_2CH_2CON(Me)$-CH_2CO-LCA-). The loading was done according to a literature procedure.[179] First a mixture of 5'-O-DMT-thymidine, CPG with a sarcosinyl-succinoyl linker, 4-DMAP, triethylamine, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (DEC) and anhydrous pyridine was shaken at room temperature for 24 hours. Then pentachlorophenol was added, and the mixture was shaken for an additional period of 16 hours. The resulting CPG was treated with reagent grade piperidine for 10 minutes, and unreacted sites capped

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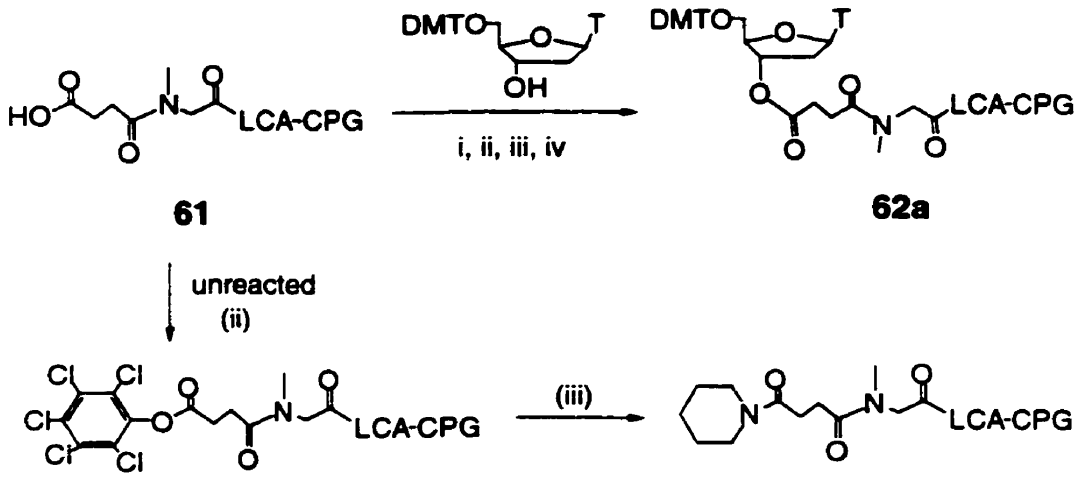
[176] The enzyme digestion was carried out in ISIS Pharmaceuticals (Carlsbad, CA).
[177] Brown, T.; Pritchard, C. E.; Turner, G.; Salisbury, S. A. *J. Chem. Soc. Chem. Commun.* **1989**, 891.
[178] Lehmann, C.; Xu, Y.-Z.; Christodoulou, C.; Tan, Z. K.; Gait, M. J. *Nucleic Acids Res.* **1989**, *17*, 2379.
[179] Damha, M. J.; Giannaris, P. A.; Zabarylo, S. V. *Nucleic Acids Res.* **1990**, *18*, 3813.

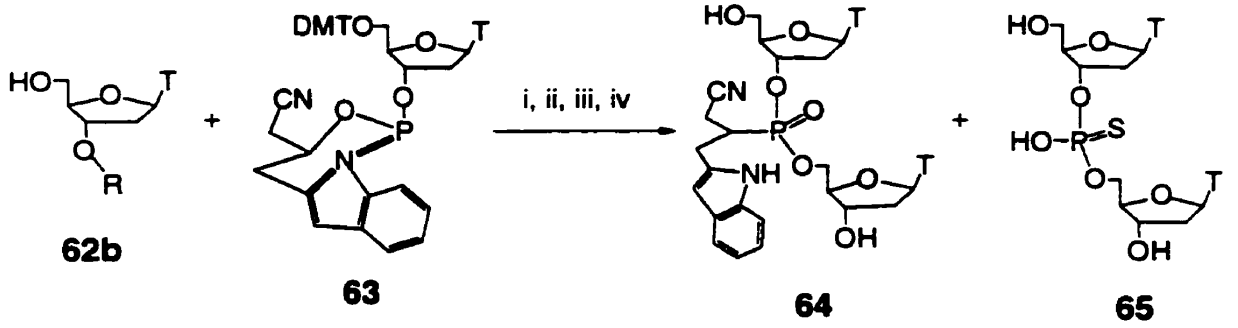
with acetic anhydride to give immobilized thymidine **62**. The loading amount was measured by Trityl Analysis, 37.9 μmol/g.



i). 4-DMAP, DEC, anhydrous pyridine. ii). Pentachlorophenol. iii) piperidine. iv) 0.5 M acetic anhydride in THF, 0.5 M 4-DMAP/2,4,6-trimethylpyridine in THF.

Scheme 27. The Loading of Thymidine on Solid Support

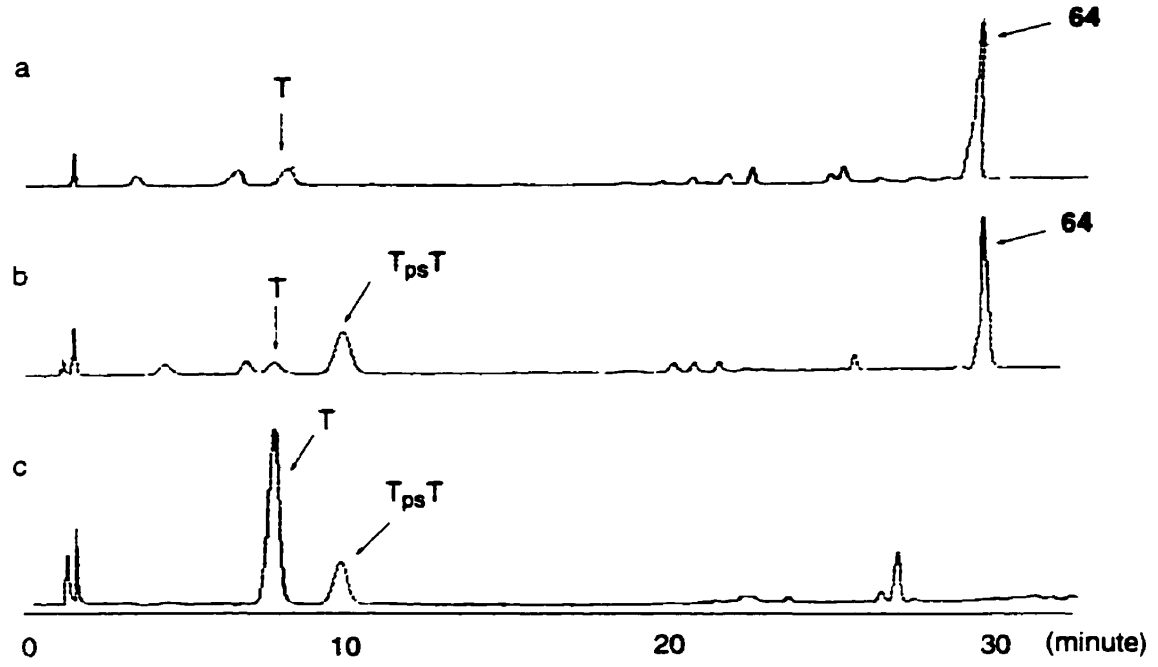
The synthesis of monomer dimethoxytrityl Sp-**63** was carried out using the same procedure as for its silylated analogue **56**. The coupling reactions were carried out manually on a solid support. To a sintered glass funnel was added thymidine **62** (1 μmol), followed by 0.2 ml of monomer Sp-**63** in acetonitrile (0.1 M, 20 μmol) and 30 μl of DBU (0.2 mmol). After five minutes, the solid support was washed with acetonitrile, sulfurized with Beaucage's reagent, and treated with aqueous ammonia.



R = $COCH_2CH_2CON(Me)CH_2CO$-LCA-CPG

Scheme 28. The Results from Solid Phase Reaction

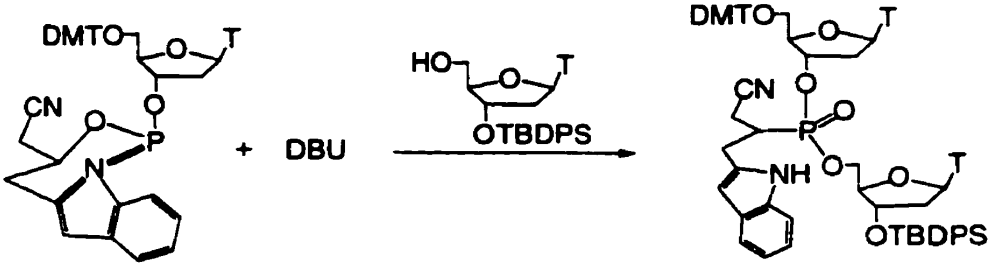
To our surprise, alkyl phosphonate **64** was obtained instead of the expected phosphorothioate $T_{ps}T$ dimer Rp-**65**. Phosphonate **64** was characterized by HPLC-MS, and the intensities of its isotopic masses perfectly matched the theoretical ones.



Scheme 29. The HPLC analysis for the reaction of thymidine **62** with **63**. HPLC: HP 1090 Series II; Waters C4 column (3.9 x 300 mm); Solvent A: water; B: acetonitrile; 1.5 ml/min flow rate; 3% B increase linearly to 7% B for the first 15 minutes, then increase to 40% B during the next 20 minutes. a. As described in text. b. To the sintered glass funnel was added thymidine **62** (1 μmol) and 30 μl of DBU (0.2 mmol) in 0.1 ml acetonitrile, then a solution of compound **63** in acetonitrile (0.2 ml, 0.1 M, 20 μmol) was added by a syringe. After five minutes, the solid support was washed with acetonitrile (3 x 2 ml) and sulfurized with Beaucage's reagent (0.1 ml, 0.1 M in THF). After detritylation, the solid support was cleaved with NH_4OH (28%) at 50°C for two hours. c. To the sintered glass funnel was added **62** (1 μmol) and 30 μl of DBU (0.2 mmol) in 0.1 ml acetonitrile. After three minutes, the solid support was washed with acetonitrile (2 x 1 ml), then a solution of compound **63** in acetonitrile (0.2 ml, 0.1 M, 20 μmol) was added by a syringe. The following is same as in procedure b.

We could get a small amount of $T_{ps}T$ dimer Rp-**65** if polymer supported thymidine **62** was first mixed with DBU, followed by addition of **63** in acetonitrile. As shown in the Scheme 29, all thymidine **62** reacted, and phosphonate **64** was formed as the major product, in addition to 10-20% of the desired $T_{ps}T$ dimer **65**. Finally, when the above procedure was repeated but the solid support washed with acetonitrile before addition of a solution of **63**, 10-20% of the desired $T_{ps}T$ dimer **65** was obtained, in addition to unreacted starting material. No phosphonate **64** was detected in this run (Scheme. 29c). By repeating the procedure c four times before sulfurization, the ratio of dimer $T_{ps}T$ to unreacted thymidine T increased from 0.30 : 1 to 1.06 : 1.

These results can be interpreted as follows: a) Reaction of polymer supported **62** with monomer **63** activated by DBU is slow as compared to DBU induced β-elimination of **63** to **67**. b) Equilibration of CPG-bound thymidine **62** with DBU probably binds a certain proportion of the DBU on the solid support, providing 10-20% of activated **62**. This activated **62** is responsible for the formation of 10-20% of the desired $T_{ps}T$ found both in runs b and c (Scheme 29).



63 **66**

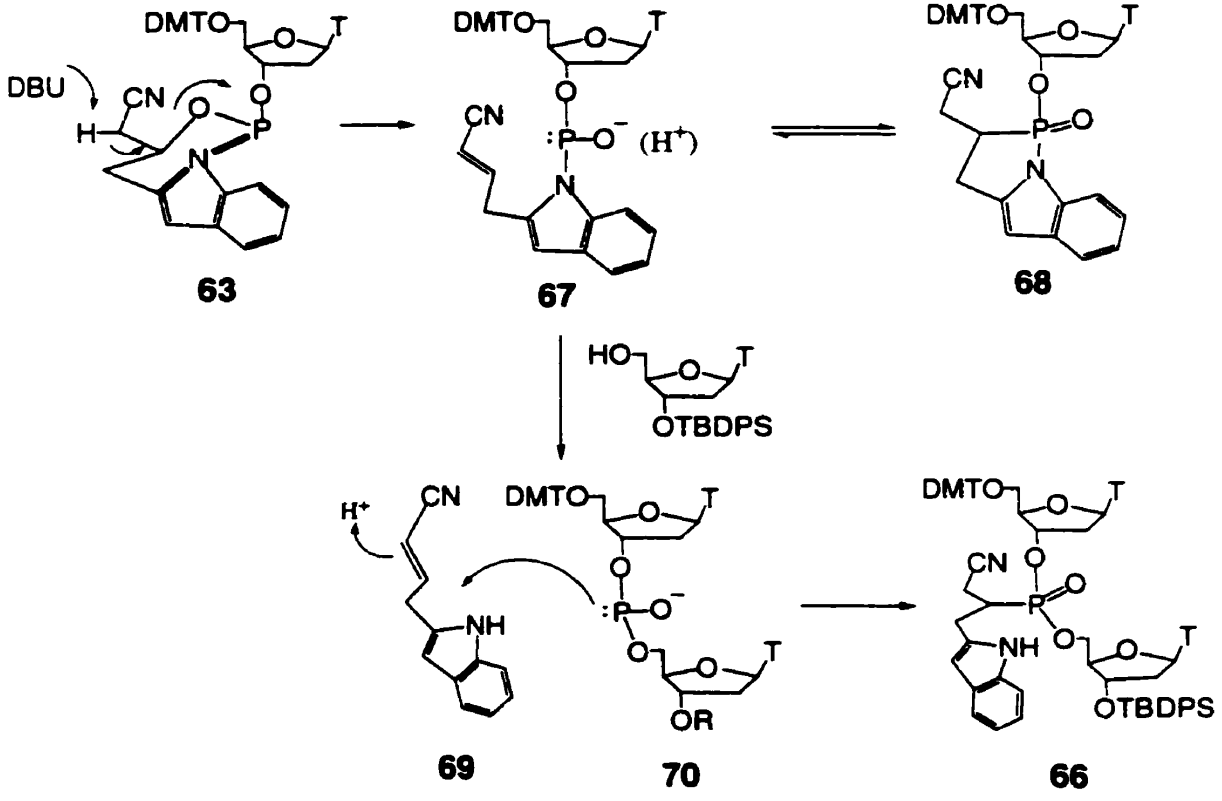
Scheme 30. The Synthesis of Alkylphosphonate **66** in Solution Phase

In order to clarify the reaction path leading to phosphonate **64**, the reaction was carried out in solution. First, 5 eq. of DBU was added to a $CDCl_3$ solution of Sp-**63**/Rp-**63** (12:1). After several minutes, two peaks in ^{31}P-NMR were observed at 32.42 ppm and 32.24 ppm in a ratio of 1 : 5. Then 1 eq. of 3'-O-TBDPS-thymidine ($T^{5'}OH$) was added. A major peak at slightly lower field appeared, and the peaks around 32 ppm disappeared. After washing with water and purification by chromatography, alkyl phosphonate **66** was obtained as a mixture of four diastereoisomers, with ^{31}P-NMR at 29.95 ppm, 29.71 ppm,

28.98 ppm, and 28.87 ppm. The ratio of these four peaks was 13.5 : 1.3 : 1.0 : 1.9. The intermediates corresponding to 32 ppm in the ^{31}P-NMR could not be isolated.

In a parallel run, Rp-**63**/Sp-**63** (7.5:1) was reacted with DBU to give two intermediates with ^{31}P NMR at 33.76 ppm and 33.34 ppm in a ratio of 4.8 : 1. After reaction with thymidine, alkylphosphonate **66** was obtained as a mixture of 4 isomers. The ratio of these four peaks in ^{31}P NMR at 29.88 ppm, 29.66 ppm, 28.97 ppm, 28.79 ppm was 8.6 : 1.0 : 12.7 : 38.2. The configuration for these compounds have not been elucidated.

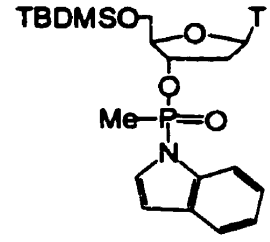
The phosphonate formation is best explained by postulating a β-elimination to form phosphite **67** or its anion, followed by formation of **68**, 32 ppm.



Scheme 31. A Possible Mechanism of the Formation of Alkylphosphonate **66**

We first thought that reaction of **68** with thymidine derivative **62** would then directly provide phosphonate **64** and hence **66**. However, the analogous phosphonate **91** did not react with 3'-O-TBDPS-thymidine ($T^{5'}OH$) under the conditions used. We therefore think that **68** is in equilibrium with **67**, which then reacts with thymidine **62** or

$T^{5'}OH$ as outlined to provide phosphite **70** or its anion. The latter then adds to the unsaturated nitrile **69** to give phosphonates **66**.



91

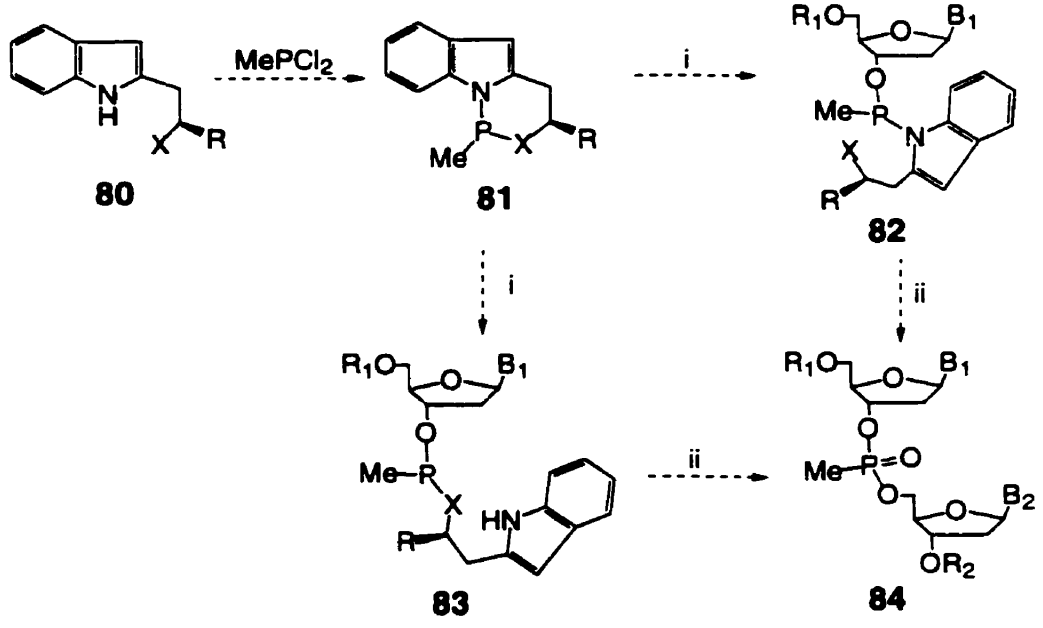
Although four diastereomeric phosphonates **66** formed, one diastereomer predominated both when Sp-**63** and Rp-**63** were used as starting materials. This reaction might be developed to a method for stereoselective synthesis of alkylphosphonates.

Chapter III. The Stereoselective Synthesis of Methylphosphonates

3.1. Introduction

We have demonstrated that indole is a good leaving group in the stereoselective synthesis of phosphorothioates. The most valuable properties for indole are that it can form a stable trivalent phosphorus compounds, and can be replaced by nucleosides in a stereospecific manner. We planned to extend its usefulness to the synthesis of methylphosphonates, especially for their stereoselective synthesis.

For the synthesis of methylphosphonates, a common and simple procedure is to use dichloromethylphosphorine ($MePCl_2$) as the starting material. Unlike phosphorus trichloride (PCl_3), there are only two reactive sides available in $MePCl_2$. Our strategy for a stereoselective synthesis of methylphosphonates was to search a chiral auxiliary like **80**



i). 3'-hydroxyl-nucleoside. ii). a. 5'-hydroxyl-nucleoside. b. I_2/H_2O

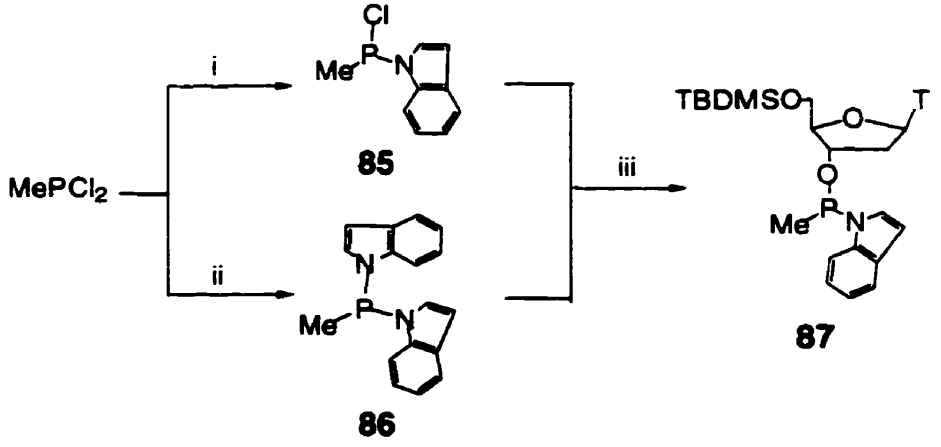
Scheme 32. A Possible Approach for Stereoselective Synthesis of Methylphosphonates

which contained two leaving groups, one an indole and the other an as yet undefined group X.

This X group which we look for should also be stereospecifically replaceable by a nucleoside. An approach for a stereoselective synthesis of methylphosphonates is shown in Scheme 32. If a diastereomerically enriched indole derivative **81** could be formed by the reaction of chiral auxiliary **80** with $MePCl_2$, we could use 3'-hydroxyl-nucleoside and 5'-hydroxyl-nucleoside to substitute the two leaving groups indole and X one by one to form a methylphosphonate **84**.

3.2. Synthesis of Methylphosphonates by Using Indole as a Leaving Group

In order to explore the pathways outlined in Scheme 32, dichloromethyl-phosphorine was first reacted with one equivalent of indole in the presence of triethylamine at 0 °C. After several minutes, the peak for $MePCl_2$ at 196 ppm in the ^{31}P NMR totally



i) Indole (1 eq.), Et_3N/CH_2Cl_2 . ii) Indole (2 eq.), Et_3N/CH_2Cl_2 . iii) 5'-O-TBDMS-thymidine.

Scheme 33. The Synthesis of Coupling Reagent **87**

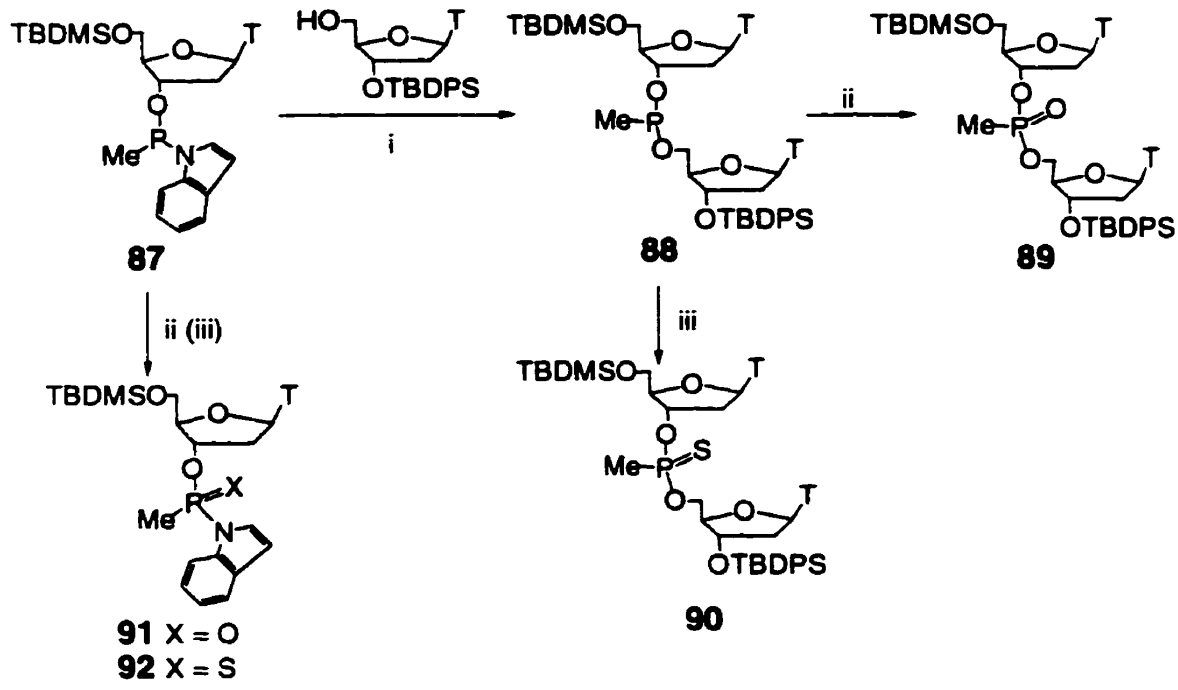
disappeared, and a new peak was observed at 112 ppm, corresponding to the formation of chloro derivative **85**. Then one equivalent of 5'-O-TBDMS-thymidine ($T^{3'}OH$) was introduced to the reaction mixture, and the chloro derivative **85** was immediately transformed to indole derivative **87**. After filtering out triethylammonium chloride and drying in vacuum , two diastereoisomers of **87** were obtained in quantitative yield, as established by ^{31}P NMR (130.3 ppm, 129.1 ppm). The product **87** did not need further purification and could be stored under argon for a long time.

When dichloromethylphosphorine reacted with two equivalents of indole, indole derivative **86** (^{31}P NMR, 47 ppm) was obtained. One indole group of **86** could be replaced by an alcohol in the presence of triethylamine to form **87**. while the indole group in **87** could not be replaced by alcohols using triethylamine as a base.

The coupling reaction of **87** with 3'-O-TBDPS-thymidine ($T^{5'}OH$) was carried out in the presence of DBU. By using two equivalents of DBU, the coupling reaction was complete to form two diastereoisomers of methylphosphinite **88** (^{31}P NMR, 185.5 ppm, 184.7 ppm) within half an hour at 50 ^{0}C. Increasing the amount of DBU accelerated the coupling reaction. The methylphosphinite **88** could be efficiently oxidized to form methylphosphonate **89** by iodine/water. With the Beaucage's reagent, methylphosphinite **88** gave methylthiophosphonate **90**. The two diastereoisomers of **89** and **90** could be separated by flash chromatography on silica gel. The whole procedure starting from dichloromethylphosphorine to phosphonate **89** or **90** could be done in one flask with an overall yield above 91%.

Oxidation of **87** with iodine or sulfur afforded the indole derivative **91** or **92**. The two diastereomers of **91** could be easily separated by silica gel column chromatography. Unlike the p-nitrophenoxy group,[148] the indole group in these pentavalent phosphorus compounds **91** and **92** was stable, and could not be replaced by alcohols in the presence of DBU or a Grignard reagent.

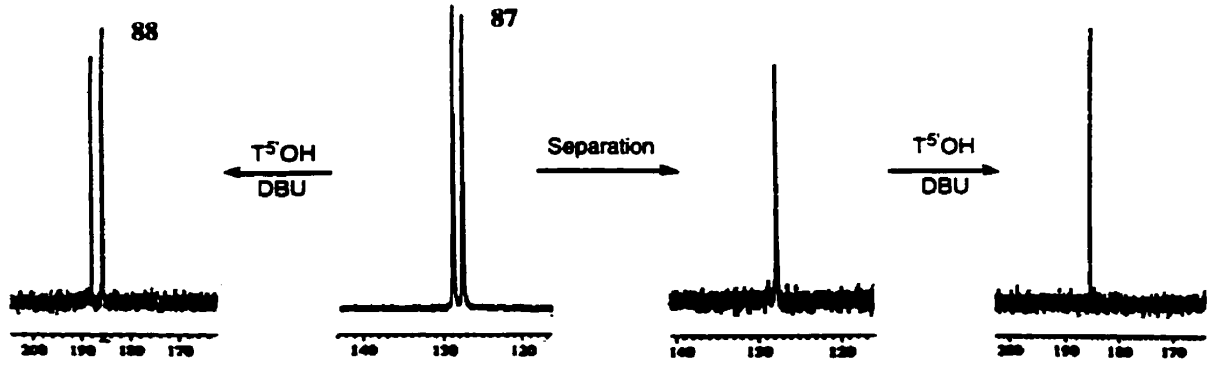
The displacement of indole group of **87** is stereospecific. It is difficult to separate two diastereoisomers of **87** by silica gel chromatography because they are easily oxidized on silica gel.



i) $T^{5'}OH$, DBU. ii) 0.1M iodine in THF-pyridine-H_2O (4:3:3 v/v). iii) Beaucage's reagent.

Scheme 34. The Synthesis of Dinucleotide Methylphosphonates

By using a TLC plate (Kieselgel 60 F_{254} glass backed plates, 0.5 mm thickness), a small amount of one diastereoisomer **87** was separated and reacted with 5'-O-TBDMS-thymidine in the presence of DBU to afford only one diastereoisomer **88** as established by ^{31}P NMR. Scheme 35 shows the appropriate ^{31}P NMR spectra.

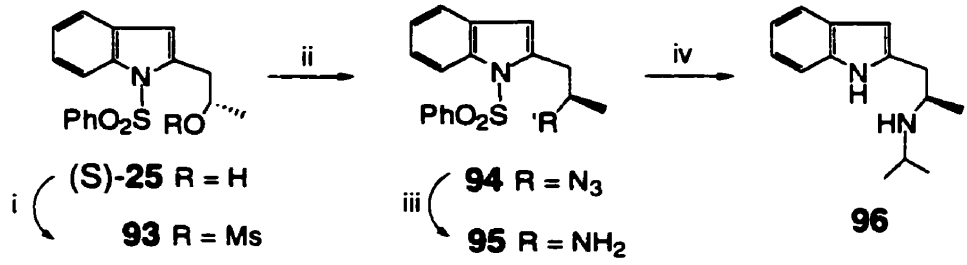


Scheme 35. The ^{31}P NMR spectra of **87** and **88**.

Here we demonstrated that the trivalent phosphorus compound containing a indole group is stable, and the DBU mediated substitution of the indole group by the other nucleoside is stereospecific. Indole therefore fits all the requirements for the chiral auxiliary **80** in our proposed approach (Scheme 32). Our next step was to search for a chiral auxiliary for the stereoselective synthesis of methylphosphonates.

3.3. The Synthesis of Chiral Auxiliaries for Stereoselective Synthesis of Methylphosphonates

Previous work has shown that by using dicyano bromoimidazole as a catalyst, amine group on phosphoramidite could be replaced by a nucleoside with some degree of stereocontrol.[137] We therefore considered to synthesize chiral auxiliary **96**, which contains an amine group.

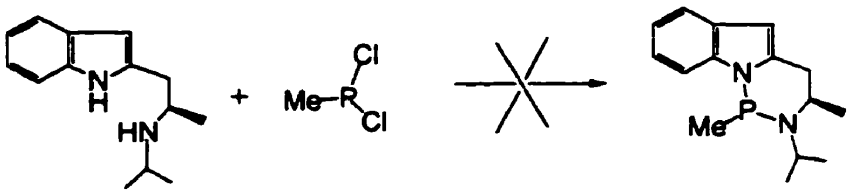


i). MsCl, Et_3N, CH_2Cl_2. ii). LiN_3, DMF. iii). H_2/Pd-C. iv). a. Acetone, $NaBH_3CN$, CH_3OH. b. KOH, CH_3OH/H_2O (3:1).

Scheme 36. The Synthesis of Amine Derivative **96**

The synthesis of amine derivative **96** started from alcohol (S)-**25**. First the hydroxyl group of **25** was mesylated, and the mesylated **93** was transformed to an azide derivative **94** with lithium azide in DMF. After hydrogenation and reductive alkylation with acetone, amine derivative **96** was obtained. However, amine **96** did not form a clean cyclic intermediate **97** with $MePCl_2$. The reaction of **96** with $MePCl_2$ gave several peaks around 40-72 ppm in ^{31}P NMR, which did not turn to one even after a week. The use of

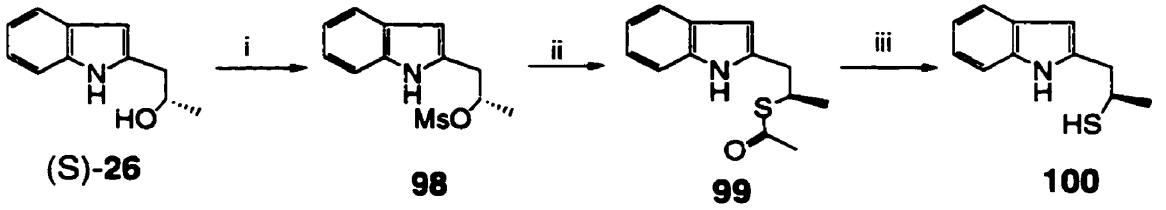
amino indole **96** for the stereoselective synthesis of methylphosphonates was therefore abandoned.



96 **97**

Scheme 37.

Stec and co-workers have reported that the thiol group in pentavalent phosphorus compound could be stereospecifically replaced by a nucleoside.[134] We therefore prepared a thiol derivative **100**. Thiol **100** was prepared from hydroxyindole (S)-**26**. The hydroxyl group was first mesylated, and the mesylated **98** was treated with potassium thioacetate to form thioacetate **99**. Ammonolysis afforded thiol **100**. Thiol **100** also could be prepared from **26** by a Mitsunobu reaction.[180] The purification of the Mitsunobu reaction mixture was difficult because a large amount of Ph_3P and DIAD had to be used.



(S)-**26** **98** **99** **100**

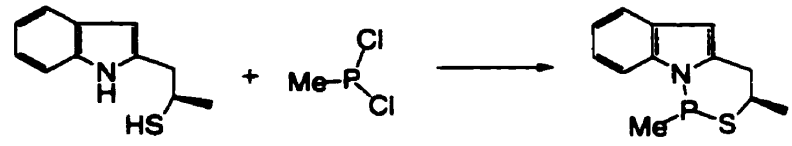
i). MsCl, Et_3N, CH_2Cl_2. ii). $KSCOCH_3$, DMF, 100 °C. iii). NH_3, CH_3OH.

Scheme 38. The Synthesis of Thiol Compound **100**

The thiol **100** was then reacted with $MePCl_2$ in the presence of triethylamine. A cyclic intermediate **101** was formed as established by ^{31}P NMR, but two peaks were observed around 115 ppm in a ratio of less than 3:1 (the two peaks were too close to be integrated separately), corresponding to the formation of two diastereomers of **101**. When 5'-O-TBDMS-thymidine was introduced, the peaks around 115 ppm disappeared and two new peaks were observed at 134.95 ppm and 135.11 ppm in a ratio of less than 3:1. Since

[180] Mitsunobu, O. *Synthesis* **1981**, 1.

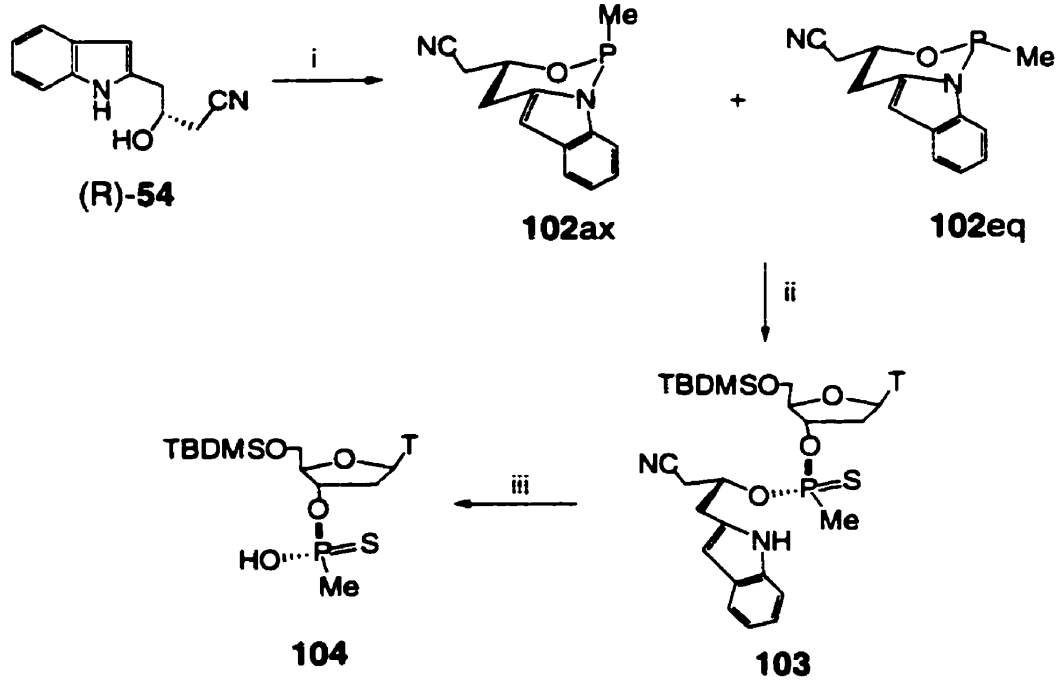
the ratio of two diastereomers of **101** could not be improved, the thiol **100** was not a good candidate for the stereoselective synthesis of methylphosphonates.



100 **101**

Scheme 39. The Reaction of thiol **100** with $MePCl_2$

We had demonstrated that cyano alcohol **54** could form a diastereomerically enriched intermediate with PCl_3. We therefore considered to react it with $MePCl_2$.



(R)-**54** **102ax** **102eq**

104 **103**

i). $MePCl_2$, THF, Et_3N (2.2 eq.), 0 °C, ii). a. T^3OH, DBU, 20 minutes. b. Beaucage's reagent. iii) NH_3, CH_3OH, RT, 0.5 hr.

Scheme 40. The Synthesis of Monoester **104**

Equimolar THF solutions of (R)-**54** and dichloromethylphosphorine ($MePCl_2$) were allowed to react at 0 °C in the presence of triethylamine, and the reaction was followed by ^{31}P NMR. After a few minutes, two peaks appeared around 135 ppm in a ratio of 6:1,

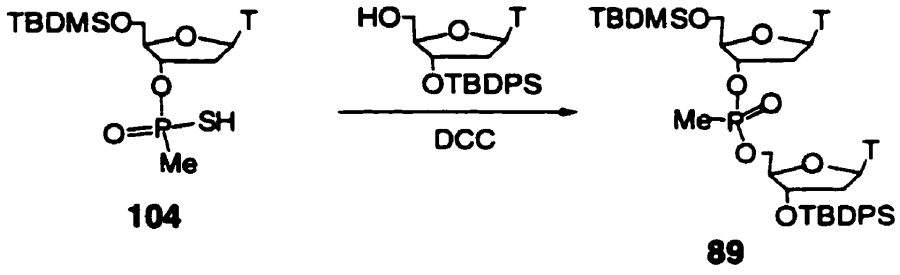
which indicated the formation of indole derivative **102**, as shown in Scheme 38. In this reaction, using THF as solvent is necessary. If acetonitrile or dichloromethane was used as the solvent, two peaks around 135 ppm were observed in a ratio of approximately 1:1. Then 5'-O-TBDMS-thymidine ($T^{3'}OH$) was added followed by 2 eq. of DBU. After the reaction mixture was stirred for 30 minutes, Beaucage's reagent was added to provide diesters **103** as a mixture of two diastereomers, the major one at 96.96 ppm and the minor one at 98.46 ppm in a ratio of 6:1. The ratio of the two diastereomers **103** could not be improved during this coupling reaction.

The chiral auxiliary on **103** was easily removed in half an hour by ammonia in methanol. Chromatographic purification on silica gel (ethyl acetate to acetone/methanol 4:1) afforded monoester **104**, ^{31}P NMR, 77.93 ppm (major) and 78.13 ppm (minor) in a ratio of 5:1.

In a parallel run, (S)-**54** provided two isomers of monoester **104**, ^{31}P NMR 76.28 ppm (minor) and 76.70 ppm (major) in a ratio of 1:5, *via* methylthiophosphonates **103** (^{31}P NMR, 97.82 ppm and 98.30 ppm in a ratio of 6:1)

3.4. The Synthesis of Methylphosphonate Diesters

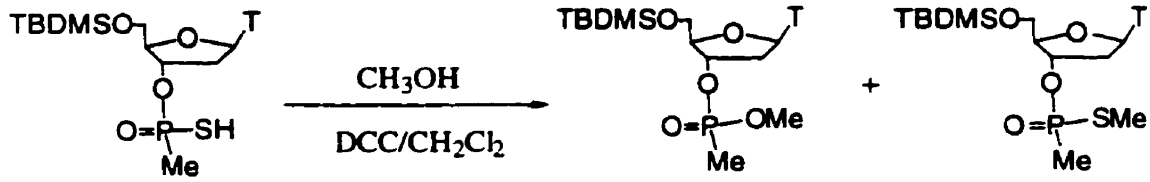
Using 1,3-dicyclohexylcarbodiimide (DCC) as the coupling reagent, the reaction of monoester **104** with $T^{5'}OH$ gave dithymidinyl methylphosphonate **89** as established by ^{31}P NMR, and the reaction is stereospecific. However, this DCC mediated reaction was very slow and afforded some side products with a ^{31}P NMR around 85 ppm. In the presence of 5 eq. of DCC, the reaction was complete at 50 ^{0}C after 4 days.



Scheme 41. The Synthesis of Methylphosphonate

Using triisopropylbenzenesulfonyl chloride (TPSCl),[181] or diethyl phosphorochloridate (DECP)[182] as the coupling reagent, we only got side products with ^{31}P NMR around 85 ppm.

The DCC mediated condensation of **104** with methanol was complete overnight and afforded methylphosphonate **105** (^{31}P NMR, 32.87 ppm, 32.76 ppm). If more than 3 eq. of methanol was used, a side product **106** (^{31}P NMR, 57.17 ppm, 56.36 ppm) became the major product.



104 **105** **106**

Scheme 42. The Reaction of **104** with Methanol

Here we demonstrated that indole is a good leaving group in the synthesis of methylphosphonates, and a diastereomerically enriched monoester **104** can be synthesized by using chiral auxiliary **54**.

[181] Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'o, P. O. P. Biochemistry, 1979, 18, 5134.

[182] Zaub, R.; Stawinski, J. *J. Org. Chem.* **1996**, *61*, 6617.

Contribution to Knowledge

A novel leaving group, indole, was discovered. It can form a stable indolephosphorine and can be stereospecifically substituted by a nucleoside in the presence of DBU.

New types of chiral precursor indole-oxazaphosphorines **31**, **39**, **44**, **56** were synthesized. A methodology for the stereoselective synthesis of phosphorothioates **60** in a de > 96% was developed by the use of chiral precursor **56**. The reaction of cyano monomer **63** on solid support was investigated. A diastereomerically enriched alkylphosphonate **66** was synthesized.

A novel internucleoside coupling reagent **87** was developed for the synthesis of methylphosphonates, in which the indole group can be replaced by a nucleoside within several minutes in the presence of DBU. A diastereomerically enriched monoester **104** (66% de) was synthesized.

Chapter IV. Experimental Section

4.1. General Methods

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

^{1}H-NMR spectra were recorded on a Varian XL-200, JEOL 270 or Varian UNITY 500 spectrometer at 200, 270 and 500 MHz respectively. ^{13}C-NMR spectra were recorded on a Varian XL-300, Jeol CFP 270 or Varian UNITY 500 spectrometer at 75.4, 67.9 and 125.7 MHz. Peak assignments were made with the help of 2D-Heteronuclear Multiple Quantum Coherence (HMQC) spectroscopy. ^{31}P-NMR spectra were recorded on Jeol CFP 270, Varian XL-300, or Varian UNITY 500 at 109.4, 121.42 and 202.3 MHz using 85% H_3PO_4 as an external standard.

Low resolution chemical ionization (CI-MS), electron ionization (EI-MS) mass spectra and fast atom bombardment (FAB-MS) were obtained on an KRATOS MS 25RFA spectrometer in the direct-inlet mode. High resolution FAB mass spectra of key compounds were obtained on a ZAB 2F HS spectrometer in the direct inlet mode (Biomedical Spectrometry Unit).

Thin Layer Chromatography (TLC) was performed using Kieselgel 60 F_{254} aluminum backed plates (0.2 mm thickness). Preparative plate thin layer chromatography (pTLC) was performed using Kieselgel 60 F_{254} glass backed plates (0.5 and 1.0 mm thickness). Spot(s) were visualized by UV, or by dipping in a solution of ammonium molybdate (2.5 g) and ceric sulfate (1.0 g) in 10% v/v aqueous sulfuric acid (100 ml) followed by heating, or by dipping into a 1% (w/v) aqueous solution of ninhydrin followed by heating.

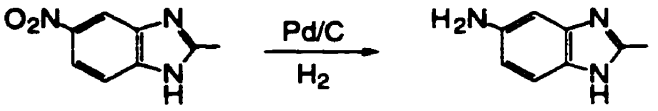
HPLC: HP 1090 Series II with Waters C-4 column (3.9 × 300 mm), Varian Vista 5500 with Chiralcel OD column (4.6 × 250 mm). Optical rotations were recorded on a Jasco DIP-140 digital polarimeter.

Tetrahydrofuran (THF) was dried by distillation on sodium benzophenone ketyl, dichloromethane on phosphorus pentoxide, methanol on magnesium, triethylamine and acetonitrile on calcium hydride, pyridine on barium oxide. Dry DMF was purchased from Aldrich Chemical Company Inc. in sure-seal bottles and was used without further drying.

Phosphorus trichloride was first degassed by refluxing for 2 hours under argon followed by fractional distillation and was stored under argon. DBU, isopropanol were purified by fractional distillation then stored over 4Å Linde molecular sieves under argon. All other chemicals were purchased from Aldrich Chemical Company Inc., Sigma Chemicals, Fluka Chemicals and were used without further purification unless specified. 3'-O-TBDPS-thymidine, solid support (CPG) and Beaucage's reagent were generously given by ISIS Pharmaceuticals (Carlsbad, CA).

4.2. Experiments for Chapter II

5-Amino-2-methylbenzimidazole 4



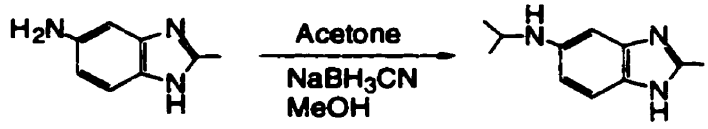
3 4

A solution of 2-methyl-5-nitrobenzimidazole (1.8 g, 10 mmol) and Pd/C (200 mg of a 10% mixture Pd/C) in 20 ml of ethanol was shaken at RT under 30 PSI of hydrogen for 8 hours. TLC showed that all the starting material **3** had been consumed. The catalyst was filtered off and washed with ethanol (5 ml). Evaporation of the solvent afforded a light yellow solid 5-amino-2-methylbenzimidazole **4** (1.5 g) in quantitative yield, m.p. 79 - 80 ^{0}C.

Two isomers were observed from its ^{1}H-NMR in a ratio of 2:1, the NH can be at 1- or 3-position.

^{1}H-NMR (270 MHz, DMSO): δ 7.34-6.40 (m, 3H, aromatic H), 3.33 (s, br., 2H, NH_2), 2.35 (s, 3H, CH_3). MS (CI, NH_3): 148 ($M+H^+$, 100%).

5-Isopropylamino-2-methylbenzimidazole 5

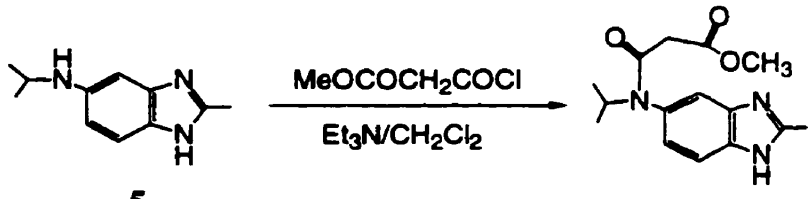


4 5

To a solution of 5-amino-2-methylbenzimidazole **4** (1.5 g, 10 mmol) in methanol (10 ml) was added 10 ml of acetone followed by sodium cyanoborohydride (1.5 g, 24 mmol). The pH of the reaction mixture was adjusted to 6 by slow addition of acetic acid and the mixture was stirred overnight at RT. The mixture was then concentrated with a rotary-evaporator, and the resulting oil was redissolved in ethyl acetate (20 ml), washed with saturated sodium carbonate (2 × 20 ml) and brine (2 × 15 ml), dried over anhydrous sodium sulfate and evaporated to give a sticky oil. This crude product was purified by flash chromatography (acetone) to give light yellow solid 5-isopropylamino-2-methyl-benzimidazole **5** (1.7 g) in 87% yield, m.p. 80 - 81 ^{0}C.

^{1}H-NMR (270 MHz, CD_3OD): δ 7.25-6.63 (m, 3H, aromatic H), 3.57 (heptet, J = 6.4 Hz, 1H, CH), 2.47 (s, 3H, CH_3), 1.19 (d, J = 6.2 Hz, 6H, $CH(CH_3)_2$). MS (CI, NH_3): 190 ($M+H^+$, 100.0%).

Benzimidazole derivative 6



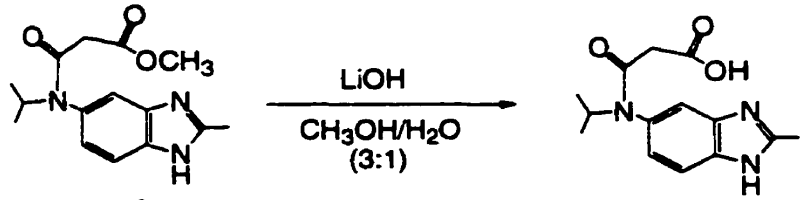
5 6

To a solution of **5** (2.0 g, 10.6 mmol) in dichloromethane (30 ml) containing 4 ml of triethylamine (28.8 mmol) was slowly added methyl malonyl chloride (1.14 ml, 10.6 mmol) at 0 ^{0}C. The mixture was stirred for 5 hours, then washed with saturated sodium bicarbonate (2 × 20 ml) and brine (2 × 20 ml), dried over anhydrous sodium sulfate and

evaporated to give a light yellow oil. Purification with flash chromatography (acetone) afforded white solid **6** (1.6 g) in 52% yield, m.p. 72 - 73 °C.

^{1}H-NMR (500 MHz, DMSO): δ 12.42 (s, br., 1H, NH), 7.47-6.88 (m, 3H, aromatic H), 4.79 (heptet, J = 6.5 Hz, 1H, CH), 3.47 (s, 3H, OCH_3), 2.79 (s, 2H, CH_2), 2.47 (s, 3H, CH_3), 0.97, 0.95 (2 x d, J = 6.5 Hz, 6H, $CH(CH_3)_2$). MS (CI, NH_3): 290 ($M+H^+$, 100.0%).

Acid derivative 7

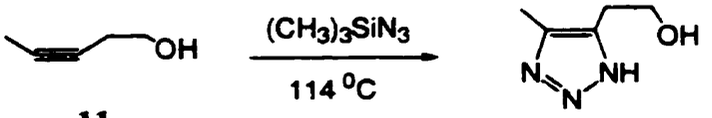


6 **7**

A solution of **6** (2.0 g, 6.9 mmol) in 40 ml methanol/water (3:1) containing 1.0 g of LiOH (23.8 mmol) was refluxed for 5 hours. Then the solution was neutralized with sulfuric acid (20%) and extracted with ethyl acetate (2 × 30 ml). The combined extracts were washed with H_2O (2 × 20 ml) and brine (2 × 20 ml), dried over anhydrous sodium sulfate, and evaporated to afford white solid acid **7** (1.8 g) in 95% yield, m.p. 139 - 140 °C.

^{1}H-NMR (270 MHz, DMSO): δ 8.41 (s, br., 1H, NH), 7.43-6.85 (m, 3H, aromatic H), 4.85 (heptet, J = 6.6 Hz, 1H, CH), 2.64 (s, 2H, CH_2), 2.47 (s, 3H, CH_3), 0.97 (m, 6H, $CH(CH_3)_2$). MS (EI): 275 ($M^{+\cdot}$, 3.6%), 259 (3.9%), 129 (48%).

4-(2-Hydroxyethyl)-5-methyl-1,2,3-triazole 12

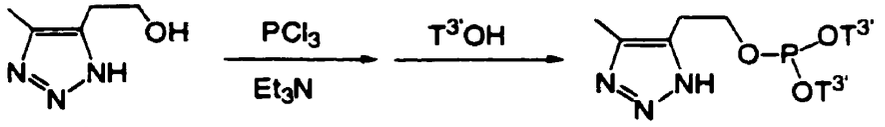


11 12

To a pressure vessel was added 1.6 g of 3-pentyn-1-ol and 3 ml of azidotrimethylsilane, then the vessel was sealed. The solution was heated at 114 °C for 5 days. Then the residue was purified by flash chromatography (hexane:ethyl acetate 1:10) to give pure white solid 4-(2-hydroxyethyl)-5-methyl-1,2,3-triazole **12** (1.80 g) in 46% yield, m.p. 96 - 97 °C.

^{1}H NMR (500 MHz, DMSO): δ 4.76 (s, broad, 1H, OH), 3.57 (t, ^3J = 7.00 Hz, 2H, CH_2O), 2.69 (t, ^3J = 7.0, 2H, CH_2), 2.16 (s, 3H, CH_3). ^{13}C NMR (67.9 MHz, DMSO): δ 142.7, 141.0, 60.9, 28.7, 10.1. MS (EI): 127 (M^+, 27.5%), 97 (M^+-CH_2O, 100.0%).

Di(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl 2-(5-methyl-1,2,3-triazol-4-yl)-ethyl phosphite 14

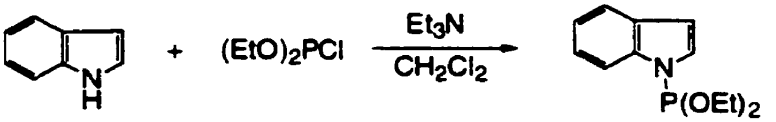


12 14

To a solution of triazole derivative **12** (0.128 g, 1.0 mmol) in CH_2Cl_2 containing 0.5 ml of triethylamine (3.6 mmol) was added 87 µl of PCl_3 at 0 °C under argon. The mixture was warmed up to 40 °C for and cooled down to 0 °C again. One eq. of 5'-O-TBDMS-thymidine ($T^{3'}OH$) was added. After the mixture was stirred for one hour, triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 × 10 ml). The solvent was evaporated to give a light yellow oil. Purification with flash chromatography (ethyl acetate) afforded phosphite triester derivative **14** (0.14 g) as light yellow solid in a yield of 16%.

^{31}P NMR (121.42 MHz, $CDCl_3$): 140.24 ppm. ^1H NMR (200 MHz, $CDCl_3$): δ 7.43 (m, 2H, 2 × H-6), 6.25 (m, 2H, 2 × H-1'), 4.85, 4.67 (m, 2H, 2 × H-3'), 4.42-3.65 (m, 10H, 2 × H-4', 2 × HH'-5', CH_2CH_2), 3.0, 2.2 (m, 4H, 2 × HH'-2'), 2.24 (s, 3H, CH_3), 1.84 (d, 6H, 2 × CH_3-5), 0.84 (s, 18H, 2 × $(CH_3)_3$), 0.10 (s, 12H, $Si(CH_3)_2$). MS (CI, NH_3): 511 (1.5%, M-$OT^{3'}$), 357 (44.3%), 281 (100.0%).

Diethyl indole-phosphorine 18

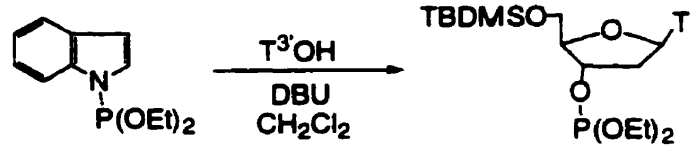


18

A 50 ml round-bottomed flask containing 1.17 g of indole (10 mmol) was dried in vacuum overnight. Under the atmosphere of argon, 20 ml of dry dichloromethane and 2 ml of triethylamine (15 mmol) were added. Then the solution was cooled to 0^0C, diethyl chlorophosphite (1.45 ml, 10 mmol) was added slowly by a syringe. As soon as the phosphite was introduced, a white precipitate was observed, corresponding to the formation of triethylammonium chloride. The mixture was stirred at room temperature for 1 hour. Then triethylammonium chloride was filtered off and washed with dry dichloromethane (2 × 10 ml). The filtrate was concentrated, passed through a short silica gel column and eluted with dry dichloromethane. The solvent was evaporated to give colorless liquid diethyl indole-phosphorine **18** (2.37 g) in quantitative yield.

^1H NMR (270 MHz, $CDCl_3$): δ 7.17 - 7.84 (m, 5H, aromatic H), 6.62 (d, 1H, ^3J = 2.7 Hz, H-3-indole), 3.90 (m, 4H, $P(OCH_2CH_3)_2$), 1.28 (t, 6H, ^3J = 6.9 Hz, $P(OCH_2CH_3)_2$). ^{31}P NMR (109.3 MHz, $CDCl_3$): 130.20 ppm.

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl diethylphosphite 19

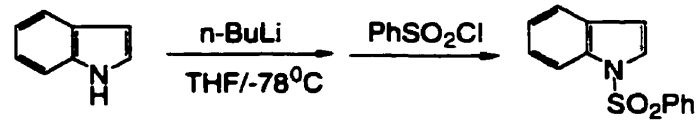


17 19

To a dry 25 ml round-bottomed flask was added 10 ml of dry dichloromethane, 59 mg of 5'-O-TBDMS-thymidine and 150 µl of DBU. Then a solution of diethyl indole-phosphorine **18** (39 mg, 0.166 mmol) in 2 ml of dry dichloromethane was added by a syringe. The mixture was stirred at room temperature for 1 hour, then evaporated to yield a light yellow oil. Purification with flash chromatography (hexane:ethyl acetate 1:1) afforded pure phosphite triester **19** as a colorless oil (76.7 mg) in 98% yield.

^{1}H NMR (270 MHz, $CDCl_3$): δ 9.71 (s, 1H, NH), 7.47 (d, 1H, ^4J = 1.2 Hz, H-6), 6.37 (dd, 1H, ^3J = 5.2, 7.2 Hz, H-1'), 5.06 (m, 1H, H-3'), 4.22 (m, 1H, H-4'), 4.08 (m, 4H, $P(OCH_2CH_3)_2$), 3.85 (m, 2H, H-5', H-5''), 2.48, 2.04 (m, 2H, H-2', H-2''), 1.29 (t, 6H, ^3J = 7.0 Hz, $P(OCH_2CH_3)_2$), 0.88 (s, 9H, $C(CH_3)_3$), 0.09 (s, 6H, CH_3SiCH_3). ^{31}P NMR (121.4 MHz, $CDCl_3$): 140.82 ppm.

1-Phenylsulfonylindole 24



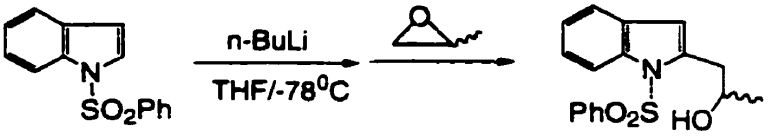
24

To a solution of indole (2.4 g, 20.5 mmol) in dry THF (20 ml) under argon at -78 °C was added dropwise *via* a syringe over 10 minutes n-butyllithium (1.6 M in hexane, 14 ml). After 30 minutes, the cooling bath was removed, and the solution was stirred for 1 hour while warming to 0 °C. The resulting indole anion precipitated as very fine white solid in a cloudy colorless solution. After the suspension was recooled to -78 °C, benzenesulfonyl chloride (2.8 ml, 22 mmol) was added neat *via* a syringe over 20 minutes.

The resulting colorless mixture was allowed to warm slowly to room temperature overnight. Then saturated NH_4Cl solution (30 ml) was added. The mixture was extracted with ethyl acetate (2 × 25 ml). The combined extracts were washed with saturated sodium bicarbonate (30 ml), water (2 × 25 ml), dried over anhydrous sodium sulfate, and evaporated to give a light amber oil which crystallized when triturated with 2:1 hexane-ether (15 ml). After standing in cold (-20 °C) for several hours, the product was collected by filtration, washed with hexane, and dried in vacuum to provide pure 1-phenylsulfonylindole **24** as white crystals (4.8 g) in 90.6% yield, m.p. 73.0 - 73.5 °C (lit.[167] m.p. 76 - 76.5 °C).

^{1}H NMR (270 MHz, $CDCl_3$): δ 7.16-7.98 (m, 9H, aromatic H), 7.63 (d, ^3J = 3.7, 1H, H-2-indole), 6.71 (d, ^3J = 3.7, 1H, H-3-indole). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 138.3, 134.9, 133.8, 130.8, 129.3, 126.8, 126.3, 124.7, 123.4, 121.5, 113.6, 109.3. MS (FAB, NBA): 258 ($M+H^+$, 85.5%), 257 (M^+, 82.1%).

(±)-(N-phenylsulfonylindol-2-yl)isopropanol 25



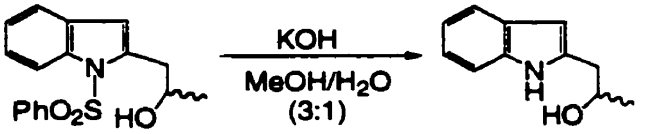
24 (±)-**25**

To a solution of 1-phenylsulfonylindole (10.3 g, 40 mmol) in dry THF (80 ml) under argon at -78 °C was added dropwise *via* a syringe over 10 minutes a solution of n-butyllithium (1.6 M in hexane, 40 mmol). The mixture was stirred for 1.5 hour below -70 °C, then allowed to warm slowly to 5 °C over 1 hour. The solution was cooled to -78 °C again, and treated *via* a syringe a solution of (±)-propylene oxide (4 ml, 57 mmol) in dry THF (10 ml). The mixture was allowed to warm slowly to room temperature overnight, poured into 1% aqueous hydrochloric acid (100 ml), and extracted with dichloromethane (3 × 100 ml). The combined extracts were washed with H_2O (2 × 50 ml) and brine (2 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford a light amber oil. Purification with flash chromatography (hexane:ethyl acetate 1:2) gave a light-amber

viscous oil which was crystallized in ether:hexane (1:1) to provide (±)-(N-phenylsulfonylindol-2-yl)isopropanol **25** as white crystals (9.19 g) in 72.8% yield, m.p. 76 - 77 °C.

^{1}H NMR (270 MHz, $CDCl_3$): δ 7.17-8.16 (m, 9H, aromatic H), 6.51 (s, 1H, H-3-indole), 4.26 (m, 1H, CHO), 3.25, 3.02 (m, 2H, CH_2), 1.93 (s, 1H, OH), 1.30 (d, ^3J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 138.8, 138.5, 137.4, 133.8, 129.7, 129.3, 126.3, 124.4, 123.9, 120.5, 115.1, 111.6, 67.2, 39.1, 23.1. MS (FAB, NBA): 316 ($M+H^+$, 66.2%), 315 (M^+, 48.2%).

(±)-indol-2-ylisopropanol 26

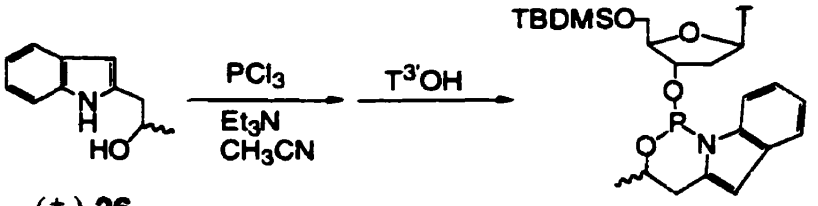


(±)-**25** (±)-**26**

A solution of (±)-(N-phenylsulfonylindol-2-yl)-isopropanol **25** (5.0 g, 15.9 mmol) containing 4.5 g of potassium hydroxide (80.4 mmol) in 50 ml of methanol:water (3:1) was refluxed for 5 hours and extracted with ethyl acetate (2 × 50 ml). The combined extracts were washed with H_2O (2 × 50 ml) and brine (2 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford pure (±)-indol-2-ylisopropanol **26** as a light amber oil (2.72 g) in 98% yield.

^{1}H NMR (270 MHz, $CDCl_3$): δ 8.52 (s, br., 1H, NH), 7.57-7.05 (m, 4H, aromatic H), 6.27 (m, 1H, H-3-indole), 4.10 (m, 1H, CHO), 2.93, 2.76 (m, 2H, CH_2), 1.25 (d, ^3J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.6, 136.2, 128.6, 121.3, 120.0, 119.7, 110.7, 100.9, 68.0, 37.5, 23.3. MS (FAB, NBA): 176 (5.3%, $M+H^+$), 175 (47.0%, M^+), 130 (100%, $M-C_2H_4O$).

Indole-oxazaphosphorine 28

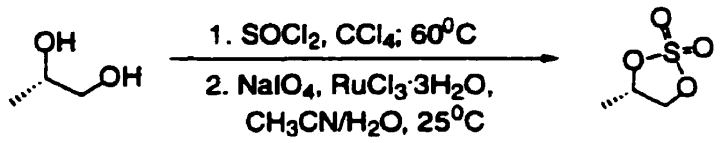


To a dry NMR tube was added (±)-indol-2-ylisopropanol **26** (50 mg, 0.286 mmol), acetonitrile (1.5 ml) and triethylamine (140 µl, 1.0 mmol). The tube was flushed with argon and sealed with a septum. Then it was cooled to 0 ^{0}C, and 24.93 µl of PCl_3 was introduced *via* a micro-syringe. The ^{31}P NMR was recorded during the reaction. The mixture was warmed up to 60 ^{0}C overnight, then cooled to 0 ^{0}C and a solution of 5'-O-TBDMS-thymidine ($T^{3'}OH$) (101.7 mg, 0.286 mmol) in 1.0 ml of CH_2Cl_2 was introduced. The tube was periodically shaken for half an hour. The salt of triethylammonium chloride was filtered off and the filtrate was evaporated to give a crude product **28**. Half of the crude product was purified by thin layer chromatography (Kieselgel 60 F_{254} glass backed plates, 1.0 mm thickness) with dichloromethane/acetonitrile (10:1) as developing solvent to give a pure indole-oxazaphosphorine **28** (46 mg) as light yellow solid (yield of purified compound: 28%.)

^{31}P NMR (202.3 MHz, $CDCl_3$): δ 120.72 ppm (36.0%), 120.58 ppm (52.4%), 121.58 (11.6%) ppm.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.5 (br m, 1H, NH), 7.5-7.1 (m, 5H, H-6, aromatic H), 6.36 (m, 2H, H-1', H-3-indole), 4.81, 4.71(m, 1H, H-3'), 4.41 (m, 1H, CHOP), 3.94, 3.60 (m, 2H, H-4', H-5'), 3.08 (m, 3H, H'-5', CH_2), 2.34, 2.00 (m, 2H, H-2', H-2''), 1.88 (d, 3H, CH_3C-5), 1.48 (2 × d, 3H, CH_3), 0.88 (m, 9H, $SiC(CH_3)_3$), 0.07 (m, 6H, $Si(CH_3)_2$). MS (FAB, NBA): 560 (5.8%, M+H$^+$), 559 (6.2%, M$^+$), 434 (12.5%), 339 (100.0%).

(S)-1,2-propanediol cyclic sulfate 29

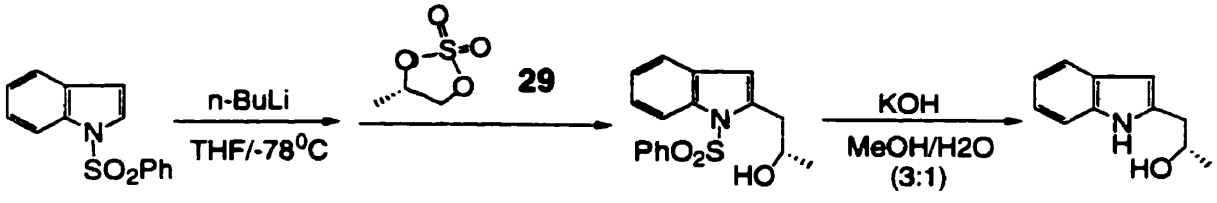


29

A 100-ml, two-necked, round-bottomed flask equipped with a reflux condenser and topped with a $CaCl_2$ drying tube connected to an HCl trap, and a rubber septum was charged with (S)-1,2-propanediol (2.3 g, 40 mmol) and CCl_4 (20 ml). Thionyl chloride (4 ml, 54.8 mmol) was added *via* a syringe, and the resulting solution was refluxed for 30 minutes. The solution was cooled with an ice-water bath and diluted with CH_3CN (20 ml). $RuCl_3 3H_2O$ (7.8 mg, 0.03 mmol) and $NaIO_4$ (12 g, 56 mmol) were added followed by water (40 ml). The resulting mixture was stirred at room temperature for 1 hour, and then diluted with ethyl acetate (150 ml). The organic solution was washed with water (30 ml), saturated aqueous sodium bicarbonate (2 × 20 ml), brine (20 ml), and dried over anhydrous sodium sulfate. The solution was filtered through a small pad of silica gel to remove the brown colored impurities. The filtrate was concentrated to afford (S)-1,2-propanediol cyclic sulfate **29** as a colorless liquid (4.0 g) in 98% yield.

^{1}H NMR (270 MHz, $CDCl_3$): δ 5.10 (ddq, $^3J_{CH2\text{-}H}$ = 8.2 Hz, 6.0 Hz, $^3J_{CH3\text{-}H}$ = 6.2 Hz, 1H, CH), 4.72 (dd, ^2J = 8.7 Hz, ^3J = 6.0 Hz, 1H, CHH'), 4.28 (dd, ^2J = 8.7 Hz, ^3J = 8.2 Hz, 1H, CHH'), 1.55 (d, ^3J = 6.2 Hz, 3H, CH3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 80.0, 74.3, 17.7.

(S)-indol-2-ylisopropanol 26



24 **(S)-25** **(S)-26**

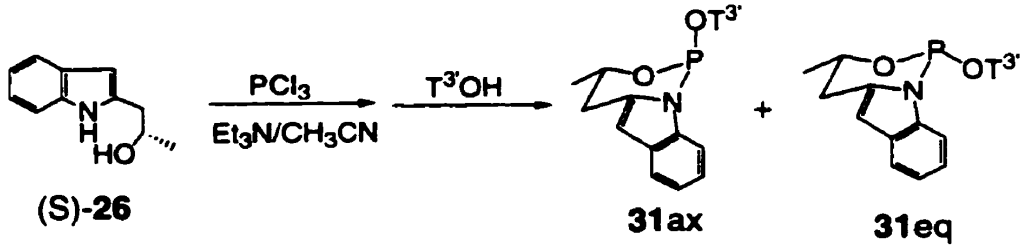
To a solution of N-phenylsulfonylindole **24** (2.57 g, 10 mmol) in dry THF (30 ml) under argon at -78 ^{0}C was added dropwise *via* a syringe 8 ml of n-butyllithium (1.6 M in hexane, 12.8 mmol) over 10 minutes. The mixture was stirred for 1.5 hour below -70 ^{0}C, then allowed to warm slowly to 5 ^{0}C over 1 hour. The solution was cooled to -78 ^{0}C again, then treated *via* a syringe a solution of (S)-1, 2-propanediol cyclic sulfate (1.5 g, 10.8 mmol) in dry THF (10 ml). The mixture was allowed to warm slowly to room temperature overnight, poured into 20% sulfuric acid (100 ml) and stirred for 3 hours. The solution was extracted with ethyl acetate (3 × 50 ml). The combined extracts were washed with H_2O (2 × 50 ml), saturated sodium bicarbonate solution (2 × 100 ml) and brine (2 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to give a light amber oil. This oil was crystallized in ether:hexane (1:1) to provide (S)-(N-phenylsulfonylindol-2-yl)isopropanol **25** as white crystals (2.85 g) in 90% yield, m.p. 88 - 89 ^{0}C. $[\alpha]^D_{295}$ - 56.11^0 (c 0.875, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): δ 7.17-8.16 (m, 9H, aromatic H), 6.51 (d, ^{4}J = 0.76, 1H, H-3-indole), 4.26 (m, 1H, CHO), 3.25, 3.01 (m, 2H, CHH'), 1. 91 (s, broad, 1H, OH), 1.30 (d, ^{3}J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 138.8, 138.5, 137.4, 133.8, 129.7, 129.3, 126.3, 124.4, 123.9, 120.5, 115.1, 111.6, 67.2, 39.1, 23.1. MS (CI, NH_3): 316 ($M+H^+$, 29.4%), 298 (11.6%), 271 (40.4%).

A solution of 2.85 g of (S)-(N-phenylsulfonylindol-2-yl)isopropanol **25** (9.0 mmol) and 2.5 g of potassium hydroxide (44.6 mmol) in 50 ml of methanol:water (3:1) was refluxed for 5 hours and extracted with ethyl acetate (2 × 50 ml). The combined extracts were washed with H_2O (2 × 50 ml) and brine (2 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford pure (S)-indol-2-ylisopropanol **26** as a light amber oil (1.65 g) in 95% yield. $[\alpha]^D_{295}$ 9.12^0 (c 1.035, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): δ 8.51 (s, 1H, NH), 7.58-7.05 (m, 4H, aromatic H), 6.28 (m, 1H, H-3-indole), 4.10 (m, 1H, CHO), 2.93, 2.76 (m, 2H, CHH'), 2.06 (s, br, 1H, OH), 1.25 (d, ^{3}J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.6, 136.2, 128.9, 121.3, 119.9, 119.7, 110.7, 100.9, 68.0, 37.5, 23.3. MS (CI, NH_3): 176 ($M+H^+$, 47.0%), 175 (M^+, 62.6%), 130 (100%, M-C_2H_4O).

Indole-oxazaphosphorine 31



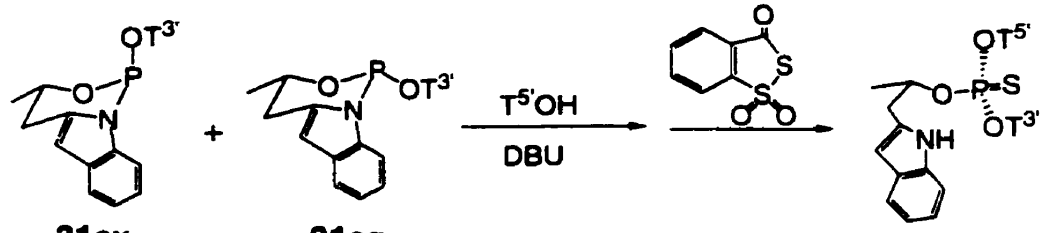
A dry 25-ml round-bottomed flask containing 10 ml of dry CH_3CN was flushed with argon and sealed with a septum. Then 100 µl of PCl_3 (1.15 mmol) was added by a micro-syringe. The flask was cooled to 0 °C in an ice-bath, and a solution of (S)-indol-2-ylpropan-2-ol **26** (200 mg, 1.15 mmol) in CH_3CN (0.35 ml) containing triethylamine (525 µl, 3.8 mmol) was introduced by a syringe. The reaction mixture was stirred for 30 minutes at 0 °C, then warmed up to 60 °C for 10 hours. The flask was cooled to 0 °C again, and a solution of 5'-O-TBDMS-thymidine (410 mg, 1.15 mmol) in CH_2Cl_2 (0.4 ml) was added. The reaction mixture was stirred at 0 °C for 30 minutes. The triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 × 10 ml). The filtrate was concentrated and purified with flash chromatography (CH_2Cl_2 : CH_3CN 1: 10) to give white solid indol-oxazaphosphorine **31** (346 mg) in 54% yield, m. p. 80 - 82 °C.

Two diastereoisomers of indole-oxazaphosphorine **31** were obtained in a ratio of 9 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 121.56 (12.4%), 120.67 (87.6%). The following NMR spectrum was assigned to the major one.

^1H NMR (500 MHz, $CDCl_3$): δ 8.81 (br s, 1H, NH), 7.39 (s, 1H, H-6), 7.54, 7.17 (m, 4H, aromatic H), 6.36 (dd, 1H, ^3J = 9.0 Hz, ^3J = 5.5 Hz, H-1'), 6.33 (s, 1H, H-3-indole), 4.72 (m, 1H, H-3'), 4.41 (m, 1H, CHOP), 3.94 (m, 1H, H-4'), 3.58 (m, 1H, H-5'), 3.06 - 3.10 (m, 3H, H-5'', CH_2), 2.36 (m, 1H, H-2'), 1.97 (m, 1H, H-2''), 1.87 (s, 3H, CH_3C-5), 1.48 (d, 3H, ^3J = 5.5 Hz, CH_3), 0.84 (s, 9H, $SiC(CH_3)_3$), -0.05 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.7 (C-4), 150.3 (C-2), 137.6, 129.8, 122.2, 121.5, 120.4, 111.1 (indole), 136.4 (C-2-indole), 135.2 (C-6), 110.6 (C-5), 103.2 (C-3-indole), 86.2 (C-4'), 86.1 (CHOP), 84.8 (C-1'), 73.7 (C-3'), 71.5 (C-5'), 62.9 (C-2'), 26.0 (CH_2), 25.9 ($SiC(CH_3)_3$), 23.0 ($SiC(CH_3)_3$), 18.3 (CH_3), 12.6 (CH_3C-

5), -5.54, -5.77 (CH_3SiCH_3). HRMS (FAB, M+H$^+$): $C_{27}H_{39}N_3O_6SiP$, Calcd. 560.234578, found 560.23459.

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl indol-2-ylisopropyl phosphorothioate 33



31ax **31eq** **33**

To a dry 5-ml round-bottomed flask was added 2 ml of dry $CHCl_3$, 50 mg of indole-oxazaphosphorine **31** (85 μmol) and 40.8 mg of 3'-O-TBDPS-thymidine ($T^{5'}OH$) (85 μmol). The flask was flushed with argon, and sealed with a septum. Then 14 μl of DBU (94 μmol) was added by a syringe. The reaction mixture was stirred at room temperature overnight and passed though a shot silica gel column to filter off DBU, and eluted with dried CH_2Cl_2/CH_3CN (1:1). The filtrate was evaporated to afford a colorless oil. The oil was redissolved in dry CH_2Cl_2 (5 ml), and Beaucage's reagent (30 mg, 1.5 mmol) was added. Evaporation of the solvent followed by flash chromatography (CH_2Cl_2/CH_3COCH_3 5:1) afforded white solid phosphorothioate triester **33** (71 mg) in 78% yield, m.p. 115 - 116 ^{0}C.

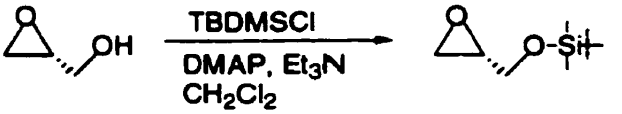
^{31}P NMR (202.3 MHz, $CDCl_3$): δ 66.76 (98.65%), 66.59(1.35%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.93 (s, 1H, NH), 9.31 (s, 1H, NH-3-$T^{5'}$), 8.85 (s, 1H, NH-3-$T^{3'}$), 7.62 - 6.93 (m, 16H, $Si(C_6H_5)_2$, C_6H_4, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.46 (dd, 1H, ^3J = 8.0 Hz, ^3J = 6.0 Hz, H-1'-$T^{5'}$), 6.26 (s, 1H, H-3-indole), 6.05 (dd, 1H, ^3J = 9.2 Hz, ^3J = 5.5 Hz, H-1'-$T^{3'}$), 4.92 (m, 1H, CHOP), 4.76 (m, 1H, H-3'-$T^{3'}$), 4.31 (m, 1H, H-3'-$T^{5'}$), 4.03 (m, 1H, H-4'-$T^{5'}$), 3.82 (m, 1H, H-4'-$T^{3'}$), 3.80, 3.50 (m, 2H, H-5', H-5''-$T^{5'}$), 3.67, 3.58 (m, 2H, H-5', H-5''-$T^{3'}$), 3.00 (m, 2H, CH_2), 2.31 (m, 1H, H-2'-$T^{5'}$), 1.94 (s, 3H, CH_3C-5-$T^{5'}$), 1.90 (s, 3H, CH_3C-5-$T^{3'}$), 1.85 (m, 1H, H-2''-$T^{5'}$), 1.60 (m, 1H, H-2'-

$T^{3'}$), 1.26 (d, 3H, ^3J = 6.0 Hz, CH_3), 1.14 (m, 1H, H-2''-$T^{3'}$), 1.80 (s, 9H, $SiC(CH_3)_3$-$T^{5'}$), 0.89 (s, 9H, $SiC(CH_3)_3$-$T^{3'}$), 0.07 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$, assigned by HMQC): δ 163.84, 163.80 (C-4-$T^{3'}$, C-4-$T^{5'}$), 150.74, 150.34 (C-2-$T^{3'}$, C-2-$T^{5'}$), 135.52, 135.49, 135.25, 134.56, 134.16, 132.73, 132.55, 130.15, 130.05, 128.48, 127.91, 127.85, 120.99, 119.59, 119.34, 111.24, 110.41 ($C_6H_5SiC_6H_5$, C_6H_5NC, C-6-$T^{3'}$, C-6-$T^{5'}$), 100.69 (C-3-indole), 85.29, 85.17 (C-4'-$T^{3'}$, C-4'-$T^{5'}$), 84.87 (C-1'-$T^{5'}$), 84.27 (C-1'-$T^{3'}$), 79.70 (C-3'-$T^{3'}$), 76.82 (CH), 73.31 (C-3'-$T^{5'}$), 66.83 (C-5'-$T^{5'}$), 63.01 (C-5'-$T^{3'}$), 40.17 (C-2'-$T^{5'}$), 37.59 (C-2'-$T^{3'}$), 36.00 (CH_2), 26.67 ($C(CH_3)_3$-$T^{5'}$), 25.77 ($C(CH_3)_3$-$T^{3'}$), 21.24 (CH_3), 18.81, 18.15 (SiC-$T^{3'}$, SiC-$T^{5'}$), 12.42, 12.33 (CH_3-$T^{3'}$, CH_3-$T^{5'}$), -5.61, -5.56 (CH_3SiCH_3). MS (FAB, NBA): 1072 ($M+H^+$, 1.3%).

The reaction of 31 with $T^{5'}OH$ followed by ^{31}P NMR — Scheme 13

A dry NMR tube containing indole-oxazaphosphorine 31 (50 mg, 89 μmol), 3'-O-TBDPS-thymidine ($T^{5'}OH$) (43 mg, 89 μmol) was dried under vacuum overnight. Then 1.5 ml $CDCl_3$ was added under argon, and the NMR tube was sealed with a septum. 10 μl trimethylphosphate was added as an internal standard. Then 1 eq. of DBU (13 μl) was introduced *via* a micro-syringe. The NMR was kept at 50 ^0C, and ^{31}P NMR was periodically recorded. The integration of the standard trimethylphosphate (4.5 ppm) was kept as 1.

(S)-glycidyl *tert*-butyldimethylsilyl ether 35



(S)-35

To a solution of (R)-glycidol (5 g, 67.5 mmol) in dry dichloromethane (40 ml) containing triethylamine (10.3 ml, 74 mmol) was added a solution of TBDMSCl (11.2 g, 74 mmol) in dry dichloromethane (30 ml) at 0 ^{0}C, and DMAP (0.33 g, 2.7 mmol). The mixture was allowed to warm up to room temperature and stirred for 5 hours. The triethylammonium chloride was filtered off and washed with dichloromethane (2 × 10 ml). The filtrate was washed with brine (2 × 50 ml), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The resulting solution was passed through a short silica gel column to remove polar impurities and eluted with hexane/ethyl acetate (3:2). After removing the solvent, a light yellow oil was collected and distilled under vacuum (50 - 56 ^{0}C/4.5 mmHg) to provide pure colorless liquid (S)-glycidyl *tert*-butyldimethylsilyl ether **35** (10.3 g) in 81.2% yield. $[\alpha]^D_{295}$ 6.11^0 (c 2.75, ethyl acetate).

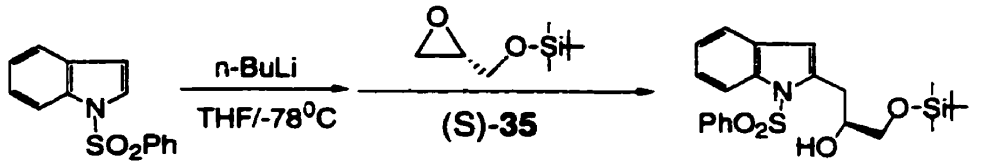
^{1}H NMR (270 MHz, $CDCl_3$): δ 3.81, 3.61 (m, 2H, CH_2OSi), 3.04 (m, 1H, CH), 2.72, 2.59 (m, 2H, CH_2O), 0.86 (s, 9H, $C(CH_3)_3$), 0.036 (d, 6H, $Si(CH_3)_2$). ^{13}C NMR (67.9 MHz, CDCl3): δ 63.78 (CH_2OSi), 52.44 (CH_2O), 44.45 (CHO), 25.90 ($(CH_3)_3$), 18.38 (CSi), -5.28, -5.32 (CH_3SiCH_3). MS (CI, NH_3): 206 ($M+NH_4^+$, 3.4%), 189 ($M+H^+$, 18.2%), 131 (78.5%), 74 (100.0%).

(R)-glycidyl *tert*-butyldimethylsilyl ether 35

Using the same procedure as for the synthesis of (S)-glycidyl *tert*-butyldimethylsilyl ether **35**, (S)-glycidol (10 g, 0.135 mol) provided a light yellow oil crude product which was distilled under vacuum (55 - 60 ^{0}C/3 mmHg) to give pure colorless liquid (R)-glycidyl *tert*-butyldimethylsilyl ether **35** (19 g) in 74.9% yield. $[\alpha]^D_{295}$ -6.09^0 (c 6.47, ethyl acetate).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.84, 3.65 (m, 2H, CH_2OSi), 3.08 (m, 1H, CH), 2.76, 2.63 (m, 2H, CH_2O), 0.88 (s, 9H, $C(CH_3)_3$), 0.07 (d, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 63.55 (CH_2OSi), 52.22 (CH_2O), 44.27 (CHO), 25.67 ($(CH_3)_3$), 18.17 (CSi), -5.51, -5.55 (CH_3SiCH_3). MS (CI, NH_3): 206 ($M+NH_4^+$, 12.1%), 189 ($M+H^+$, 12.0%), 131 (100.0%).

(S)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-iso-propanol 36



24 (S)-**36**

To a solution of 1-phenylsulfonyl-indole **24** (6.2 g, 24 mmol) in dry THF (60 ml) was added dropwise *via* a syringe 18 ml of n-butyllithium (1.6 M in hexane, 28.8 mmol) over 10 minutes under argon at -78 ^{0}C. The mixture was stirred for 1.5 hour below -70 ^{0}C, then allowed to warm slowly to 5 ^{0}C over 1 hour. The solution was cooled to -78 ^{0}C again, and a solution of (S)-glycidyl *tert*-butyldimethylsilyl ether **35** (4.5 g, 24 mmol) in dry THF (10 ml) was added *via* a syringe. The mixture was allowed to warm slowly to room temperature overnight, poured into saturated NH_4Cl solution (80 ml). The mixture was extracted with ethyl acetate (3 × 40 ml). The combined extracts were washed with H_2O (2 × 100 ml), saturated sodium bicarbonate solution (2 × 100 ml) and brine (2 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford a deep red oil. This oil was purified by silica gel chromatography (ethyl acetate/hexane 1:1) to provide (S)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-isopropanol **36** as pale light yellow solid (5.2 g) in 48.4% yield, m.p. 78 - 79.5 ^{0}C. $[\alpha]^D_{295}$ 26.88^0 (c 1.09, ethyl acetate).

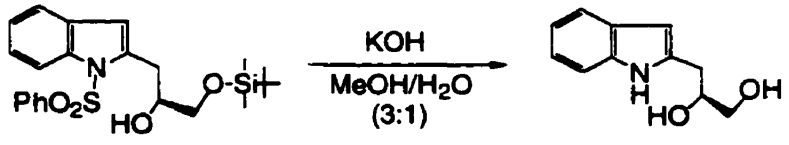
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19-8.16 (m, 9H, aromatic H), 6.57 (s, 1H, H-3-indole), 4.14 (m, 1H, CHO), 3.74, 3.58 (m, 2H, CH_2OSi), 3.23, 3.09 (m, 2H, CH_2C), 2.57 (d, 1H, ^3J = 4.5 Hz, OH), 0.93 (s, 9H, $(CH_3)_3$), 0.10 (d, 6H, CH_3SiCH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 138.76, 138.13, 137.11, 133.49, 129.64, 129.04, 126.01, 124.01, 123.55, 120.20, 114.75, 111.06 (C_6H_5, C_8H_5), 70.69 (CHOH), 66.32 (CH_2OSi), 32.84 (CH_2C), 25.72 ($(CH_3)_3$), 18.12 (CSi), -5.50, -5.54 (CH_3SiCH_3). MS (CI, NH_3): 446 ($M+H^+$, 72.4%), 388 (40.0%), 247 (80.0%), 130 (100.0%).

(R)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)iso-propanol 36

Using the same procedure as for the synthesis of (S)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)isopropanol **36**, (R)-glycidyl *tert*-butyldimethylsilyl ether **35** (14.35 g, 76.3 mmol) provided (R)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonyl-indol-2-yl)isopropanol **36** as pale light yellow solid (13.8 g) in 40.6% yield, m.p. 77 - 78.5 ^{0}C. $[\alpha]^D_{295}$ -26.90^0 (c 0.875, ethyl acetate).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20-8.16 (m, 9H, aromatic H), 6.57 (s, 1H, H-3-indole), 4.15 (m, 1H, CHO), 3.74, 3.59 (m, 2H, CH_2OSi), 3.24, 3.10 (m, 2H, CH_2C), 2.42 (s, broad, 1H, OH), 0.93 (s, 9H, $(CH_3)_3$), 0.105, 0.103 (d, 6H, CH_3SiCH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 138.76, 138.14, 137.11, 133.49, 129.64, 129.04, 126.02, 124.01, 123.55, 120.20, 114.76, 111.07 (C_6H_5, C_8H_5), 70.69 (CHOH), 66.33 (CH_2OSi), 32.84 (CH_2C), 25.71 ($(CH_3)_3$), 18.12 (CSi), -5.50, -5.54 (CH_3SiCH_3). MS (FAB, NBA): 446 ($M+H^+$, 53.5%).

(S)-3-indol-2-yl-propane-1,2-diol 37



(S)-**36** (S)-**37**

A solution of 4.5 g of (S)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-isopropanol **36** (10.1 mmol) was dissolved in 50 ml of methanol/water (3:1) containing 2.8 g of KOH (50 mmol) was refluxed for 5 hours and extracted with ethyl acetate (2 × 50 ml). The combined extracts were washed with H_2O (2 × 100 ml) and brine (2 × 100 ml), dried over anhydrous sodium sulfate, and evaporated to afford pure (S)-3-indol-2-yl-propane-1,2-diol **37** (1.68 g) as pale solid in 86.9% yield, m.p. 58.5 - 60 ^{0}C. $[\alpha]^D_{295}$ -8.53^0 (c 0.92, ethyl acetate).

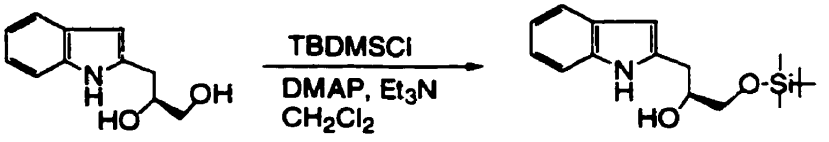
^{1}H NMR (270 MHz, $CDCl_3$): δ 8.58 (s, 1H, NH), 7.53-7.00 (m, 4H, C_6H_4), 6.21 (s, 1H, NCCH), 3.88 (m, 1H, CHO), 3.56, 3.40 (m, 2H, CH_2O), 3.2 (s, broad, 1H, OH), 2.79 (m, 2H, CH_2C), 2.00 (s, broad, 1H, OH). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.24, 135.71, 128.46, 121.45, 119.96, 119.78, 110.75 (indole), 100.93 (C-3-indole), 71.80 (CHO), 66.06 (CH_2C), 31.87 (CH_2OH). MS (CI, NH_3): 192 ($M+H^+$, 100.0%), 130 (68.6%).

(R)-3-indol-2-yl-propane-1,2-diol 37

Using the same procedure as for the synthesis of (S)-3-indol-2-yl-propane-1,2-diol **37**, removal of phenylsulfonyl and TBDMS protecting groups of (R)-1-*tert*-butyldimethylsilyloxy-3-(N-phenylsulfonylindol-2-yl)-isopropanol **36** (10 g, 22.5 mmol) with potassium hydroxide (6.0 g, 107 mmol) gave (R)-3-indol-2-yl-propane-1,2-diol **37** (4.2 g) as pale solid in 98% yield, m.p. 57.5 - 58.5 ^{0}C. $[\alpha]^D_{295}$ +10.8^0 (c 1.0, ethyl acetate).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (s, 1H, NH), 7.54, 7.26, 7.12 (m, 4H, indole), 6.21 (s, 1H, H-3-indole), 3.82 (m, 1H, CHO), 3.52, 3.34 (m, 2H, CH_2O), 3.28 (s, broad, 2H, OH), 2.72 (m, 2H, CH_2C). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 135.98, 135.48, 128.24, 121.22, 119.74, 119.59, 110.57 (indole), 100.64 (C-3-indole), 71.59 (CHO), 65.77 (CH_2OH), 31.62 (CH_2C). MS (EI): 191 ($M^{+\cdot}$, 40.8%), 130 (100.0%).

(S)-1-*tert*-butyldimethylsilyloxy-3-(indol-2-yl)-isopropanol 38

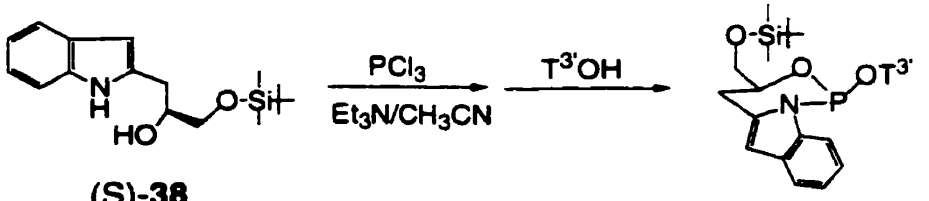


(S)-**37** (S)-**38**

To a solution of (S)-3-indol-2-yl-propane-1,2-diol **37** (1.3 g, 6.8 mmol) in dry dichloromethane (30 ml) containing triethylamine (1.1 ml, 7.9 mmol) was added a solution of TBDMSCl (1.16 g, 7.7 mmol) in dry dichloromethane (5 ml) at 0 ^{0}C, and DMAP (34.2 mg, 0.28 mmol). The mixture was allowed to warm up to room temperature and stirred for 5 hours. The triethylammonium chloride was filtered off and washed with dichloromethane (2 × 10 ml). The filtrate was washed with brine (2 × 30 ml), dried over anhydrous sodium sulfate and evaporated to give a light red oil. Purification by flash chromatography (ethyl acetate) afforded light red solid (S)-1-*tert*-butyldimethylsilyloxy-3-(indol-2-yl)-isopropanol **38** (1.57 g) in 74% yield.

^{1}H NMR (270 MHz, $CDCl_3$): δ 8.74 (br. s, 1H, NH), 7.55-7.06 (m, 4H, indole), 6.24 (s, 1H, H-3-indole), 3.99 (m, 1H, CHO), 3.65, 3.49 (m, 2H, CH_2O), 2.92(m, 2H, CH_2C), 2.74 (d, J = 3.4 Hz, 1H, OH), 0.91 (s, 9H, $C(CH_3)_3$), 0.085 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.35, 136.26, 128.43, 121.20, 119.86, 119.54, 110.64 (indole), 100.74 (C-3-indole), 71.75 (CHO), 66.54 (CH_2C), 31.51 (CH_2O), 25.95 ($(CH_3)_3$), 18.35 (SiC), -5.24, -5.27 (CH_3SiCH_3). MS (FAB, NBA): 306 ($M+H^+$, 22.0%).

Indole-oxazaphosphorine 39



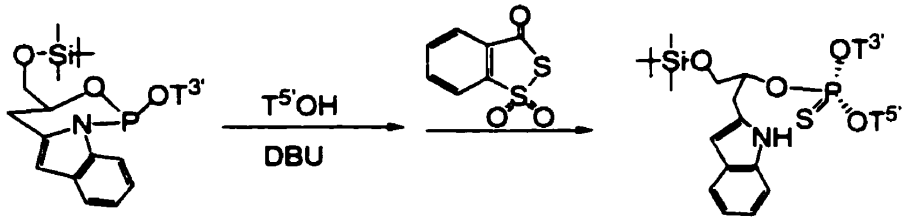
(S)-38 **39**

Using the same procedure as for the synthesis of indole-oxazaphosphorine **28**, the reaction of **38** (60 mg, 0.20 mmol) with PCl_3 and 5'-O-TBDMS-thymidine provided white solid indole-oxazaphosphorine **39** (15 mg) in 22% yield, m.p. 70 - 71 ^{0}C.

Two diastereoisomers of indole-oxazaphosphorine **39** were obtained in a ratio of 13.6 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 121.02 (6.8%), 120.64 (93.2%). The following NMR spectrum was assigned to the major one.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (br s, 1H, NH), 7.38 (d, J = 1.5 Hz, 1H, H-6), 7.54, 7.13 (m, 4H, indole), 6.36 (m, 2H, H-1', H-3-indole), 4.75 (m, 1H, H-3'), 4.42 (m, 1H, CHOP), 3.87 (m, 2H, CH2O), 3.80 (m, 1H, H-4'), 3.72 (m, 1H, H-5'), 3.16 (m, 3H, H'-5', CH_2), 2.29 (m, 1H, H-2'), 1.89 (m, 1H, H'-2'), 1.85 (d, J = 1.0 Hz, 3H, CH_3C-5), 0.90, 0.84 (2 × s, 2 × 9H, 2 × $SiC(CH_3)_3$), 0.097, 0.017 (2 × ss, 2 × 6H, 2 × $Si(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.25 (C-4), 149.82 (C-2), 137.78, 137.66, 135.62, 134.98, 129.63, 122.04, 121.28, 120.22, 110.20, 110.11, 103.38, 86.23, 84.47, 75.68, 75.62, 73.79, 73.71, 66.08, 62.70, 39.71, 28.45, 28.40, 25.71, 18.21, 18.11, 12.28, -5.45, -5.56, -5.69. MS (FAB, NBA): 690 ($M+H^+$, 2.76%), 689 (M^+, 6.21%).

Phosphorothioate triester 40

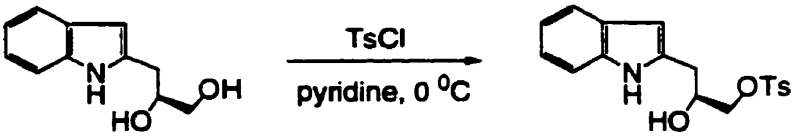


39 **40**

Using the same procedure as for the synthesis of the phosphorothioate triester **33**, 15 mg of indole-oxazaphosphorine **39** (0.022 mmol) provided 18 mg of white solid phosphorothioate triester **40** in 70.4% yield.

^{31}P NMR (109.4 MHz, $CDCl_3$): δ 69.10 ppm.

(S)-3-Indol-2-yl-2-hydroxypropyl p-toluenesulfonate 41



(S)-37 (S)-41

To a solution of (S)-3-indol-2-yl-propane-1,2-diol **37** (15 g, 78.5 mmol) in dry pyridine (120 ml) was added p-toluenesulfonyl chloride (15.5 g, 81.3 mmol) at 0 °C. After stirring for 5 hours at 0 °C, the solution was poured into 100 ml of cold hydrochloric acid (6 N) and extracted with ether (3 × 60 ml). The combined extracts were washed with hydrochloric acid (6 N, 2 × 30 ml), brine (2 × 80 ml), dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was purified by flash chromatography to give white solid (S)-3-Indol-2-yl-2-hydroxypropanyl p-toluenesulfonate **41** (24.5 g) in 90.4% yield, m.p. 96 - 97 °C. $[\alpha]^{D}_{295}$ +0.93° (c 1.0, ethyl acetate).

^{1}H NMR (500 MHz, $CDCl_3$): 8.51 (s, broad, 1H, NH), 7.78-7.06 (m, 8H, aromatic H), 6.21 (s, 1H, H-3-indole), 4.16 (m, 1H, CHO), 4.0 (m, 2H, CH_2O), 2.97 (m, 2H, CH_2C), 2.44 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 145.15, 136.09, 133.99, 132.17, 129.87, 127.78, 121.36, 119.75, 119.55, 110.56, 101.28, 72.49, 68.98, 31.27, 21.49. MS (CI, NH_3): 346 ($M+H^+$, 6.0%), 174 (100.0%).

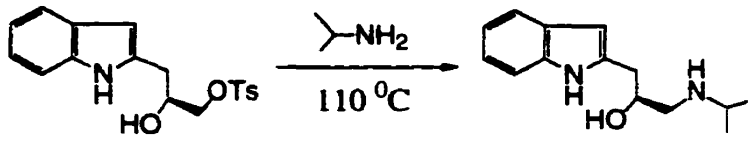
(R)-3-Indol-2-yl-2-hydroxypropyl p-toluenesulfonate 41

Using the same procedure as was outlined for the synthesis of (S)-3-Indol-2-yl-2-hydroxypropanyl p-toluenesulfonate **41**, 1.95 g of (R)-3-indol-2-yl-propane-1,2-diol **37** (10.2 mmol) provided 3.1 g of (R)-3-Indol-2-yl-2-hydroxypropanyl p-toluenesulfonate **41** as white solid in 89% yield, m.p. 112 - 113 °C. $[\alpha]^{D}_{295}$ -1.03° (c 1.035, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): 8.52 (s, broad, 1H, NH), 7.77-7.03 (m, 8H, aromatic H), 6.19 (s, 1H, H-3-indole), 4.15 (m, 1H, CHO), 3.97 (m, 2H, CH_2O), 2.92 (m, 2H, CH_2C), 2.71 (d, J = 4.5 Hz, 1H, OH), 2.42 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 145.40, 136.34, 134.29, 132.40, 130.11, 128.32, 128.02, 121.58,

119.99, 119.78, 110.83, 101.49, 72.77, 69.22, 31.54, 21.74. MS (FAB, NBA): 346 ($M+H^+$, 80.5%).

(S)-3-indol-2-yl-1-isopropylamino-isopropanol 42

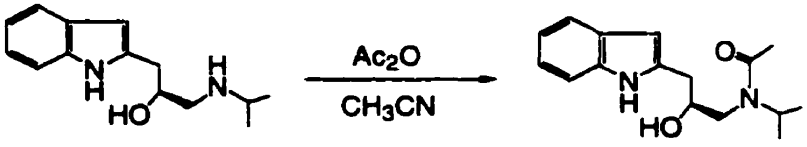


(S)-41 (S)-42

To a pressure vessel was added 2.94 g of sulfonate derivative (S)-**41** and 10 ml of isopropylamine. The mixture was stirred overnight at 110°C. Evaporation of the solvent afforded a amber oil which was purified by flash chromatography (acetone/triethylamine 10:1) to give a sticky solid (S)-3-indol-2-yl-1-isopropylamino-isopropanol **42** (1.6 g) in 81% yield. $[\alpha]^{D}_{295}$ -8.13° (c 0.75, ethyl acetate).

^{1}H NMR (270 MHz, CDCl3): 9.01 (s, broad, 1H, NH), 7.0-7.5 (m, 4H, aromatic H), 6.23(s, 1H, H-3-indole), 3.89 (m, 1H, CHO), 2.72-3.03 (m, 5H, NCH, OH, NH, CH_2), 2.44, 2.59 (m, 2H, CH_2N), 1.05, 1.04 (d, 6H, ^3J = 6.18 Hz, $(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): 136.61, 136.28, 128.43, 121.12, 119.81, 119.46, 110.73, 100.68 (C_8H_5N), 69.37, 51.80, 49.04, 33.34, 23.12, 22.81. MS (CI, NH_3): 233 ($M+H^+$, 100.0%), 130 (31.9%).

N-(3-indol-2-yl-2-hydroxy)-propyl-N-isopropylacetamide 43

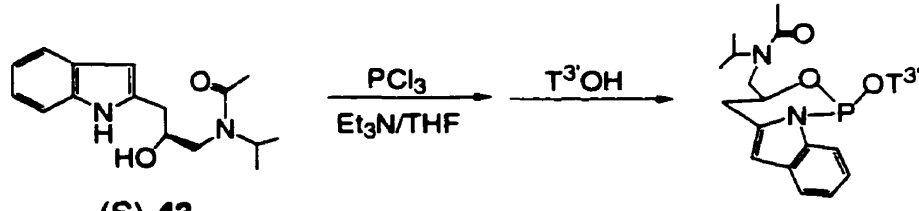


(S)-42 (S)-43

To a solution of (S)-3-indol-2-yl-1-isopropylamino-isopropanol **42** (0.2 g, 0.86 mmol) in dry CH_3CN (20 ml) was added acetic anhydride (0.1 ml, 1.06 mmol). The mixture was stirred for 4 hours at room temperature, then washed with saturated sodium bicarbonate solution (2 × 10 ml), brine (2 × 10 ml), and dried over anhydrous sodium sulfate. The solvent was evaporated and the residual solid was purified on flash chromatography (ethyl acetate) to give light yellow solid (S)-N-(3-indol-2-yl-2-hydroxy)-propyl-N-isopropyl-acetamide **43** (0.22 g) in 92% yield, m.p. 122 - 123 ^{0}C. $[\alpha]^D_{295}$ - 13.56^0 (c 0.78, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): 9.17 (s, broad, 1H, NH), 7.0-7.5 (m, 4H, aromatic H), 6.25 (s, 1H, H-3-indole), 3.98 (m, 2H, CHO, CHN), 3.50, 3.10 (m, 2H, CH_2), 2.94 (m, 2H, CH_2N), 2.15 (s, 3H, CH_3CO),1.14, 1.12 (d, 6H, ^3J = 6.42 Hz, $(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): 173.58 (CO), 136.34, 136.16, 128.24, 121.17, 119.70, 119.41, 110.89, 100.85 (C_8H_5N), 73.41, 50.15, 47.92, 34.12, 21.92, 21.32, 20.73. MS (CI, NH_3): 275 (M+H$^+$, 100.0%), 257 (75.0%), 256 (74.1%).

Indole-oxazaphosphorine 44



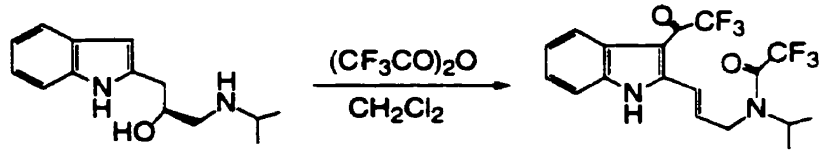
(S)-43 44

Using the same procedure as was outlined for the synthesis of indole-oxazaphosphorine **28**, the reaction of (S)-**43** (30 mg, 0.11 mmol) with PCl_3 (9.56 µl, 0.11 mmol) and 5'-O-TBDMS-thymidine (39 mg, 0.11 mmol) provided white solid indole-oxazaphosphorine **44** (42 mg) in 58% yield.

Two diastereoisomers of indole-oxazaphosphorine **44** were obtained in a ratio of 6.8 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 120.76 (12.8%), 120.68 (87.2%). The following NMR spectrum was assigned to the major one.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (br s, 1H, NH), 7.39 (s, 1H, H-6), 7.54, 7.17 (m, 4H, aromatic H), 6.35 (m, 2H, H-1', H-3-indole), 4.74 (m, 1H, H-3'), 4.46 (m, 1H, CHOP), 4.10 (m, 2H, H-4', NCH), 3.89, 3.47 (m, 3H, H-5', CH_2), 3.11 (m, 1H, H'-5', CH_2N), 2.4, 1.9 (m, 2H, HH'-2'), 2.17 (s, 3H, CH_3CO), 1.86 (s, 3H, CH_3C-5), 1.24 (m, 6H, $(CH_3)_2$), 0.87 (s, 9H, $SiC(CH_3)_3$), 0.035 (ss, 6H, $Si(CH_3)_2$).

N-3-(3'-trifluoroacetylindol-2'-yl)-2-propenyl-N-isopropyl-trifluoroacetamide 50



(S)-**42** **50**

A solution of (S)-3-indol-2-yl-1-isopropylamino-isopropanol **42** (0.8 g, 3.4 mmol) in CH_2Cl_2 (20 ml) containing trifluoroacetic anhydride (0.5 ml, 3.54 mmol) was stirred overnight. The solvent was evaporated and the crude product was purified with flash chromatography (hexanes:ethyl acetate 2:1) to give 1.0 g of light yellow solid N-3-(3'-trifluoroacetyl-indol-2'-yl)-2-propenyl-N-isopropyl-trifluoroacetamide **50** in 71% yield, m.p. 173-174 ^{0}C.

^{1}H NMR (270 MHz, CDCl3): δ 10.22 (s, broad, 1H, NH), 7.9, 7.3 (m, 4H, aromatic H), 7.44 (d, J = 16.3 Hz, 1H, CCH), 6.59 (dt, J = 16.3 Hz, 5.9 Hz, 1H, CH), 4.38 (heptet, J = 6.4 Hz, CHN), 4.22 (d, 2H, NCH_2), 1.34 (d, J = 6.6 Hz, 6H, $(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 176.37 (d), 157.50 (d), 144.50, 135.85, 131.29, 125.52, 124.66, 123.32, 122.86, 121.44, 111.56, 108.29, 49.97, 44.43, 21.31. MS (CI, NH_3): 424 ($M+NH_4^+$, 19.3%), 407 ($M+H^+$, 57.3%), 238 (100.0%).

(R)-3-hydroxy-4-(2-indolyl)butyronitrile 54

(R)-41 (R)-54

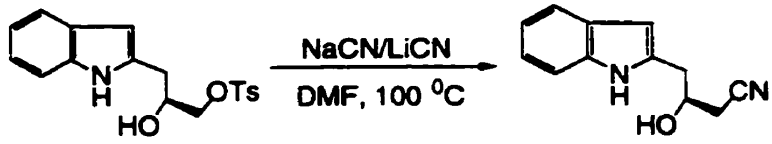
To a solution of 0.5 M LiCN in DMF (30 ml) was added (R)-3-indol-2-yl-2-hydroxypropanyl p-toluenesulfonate **41** (5.6 g, 16.2 mmol) and sodium cyanide (1.5 g, 30.6 mmol). The reaction mixture was stirred for 1 hour at 100 °C, then cooled down to room temperature, poured into 80 ml ice-water, and extracted with ethyl acetate (3 × 50 ml). The combined organic solution was washed with saturated sodium bicarbonate (2 × 50 ml), brine (2 × 30 ml), dried over anhydrous sodium sulfate, and evaporated to yield a deep red oil. This oil was purified by flash chromatography (hexane:ethyl acetate 2:3) to give light yellow solid (R)-3-hydroxy-4-(2-indolyl)butyronitrile **54** (2.4 g) in 74% yield.

The chiralty of (R)-**54** was analyzed by HPLC (Varian Vista 5500) with Chiralcel OD column (4.6 × 250 mm) in 1.5 ml/min flow rate of hexane:ethanol (9:1) and was 91% ee. (R)-**54** (2.0 g) was dissolved in chloroform (2 ml), and the solvent was slowly evaporated at room temperature in atmosphere. After a week, crystals were formed and filtered off, m.p. 92 - 93 °C.

The filtrate was collected and dried under vacuum to give (R)-**54** (0.5 g) in 96% ee. $[\alpha]^{D}_{295}$ -8.67° (c 0.565, ethyl acetate).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (s, broad, 1H, NH), 7.5-7.0 (m, 4H, aromatic H), 6.32 (s, 1H, H-3-indole), 4.22 (m, 1H, CHO), 3.05, 2.96 (m, 2H, CH_2), 2.67 (s, br. 1H, OH), 2.49 (m, 2H, CH_2CN). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 136.12, 133.37, 128.09, 121.71, 119.94, 119.81, 110.60, 101.82 (C_8H_5N), 117.21 (CN), 67.23 (CH_2C), 34.71 (CHO), 25.06 (CH_2CN). MS (EI): 200 ($M^{+\cdot}$, 48.5%), 130 (100%).

(S)-3-hydroxy-4-(2-indolyl)butyronitrile 54



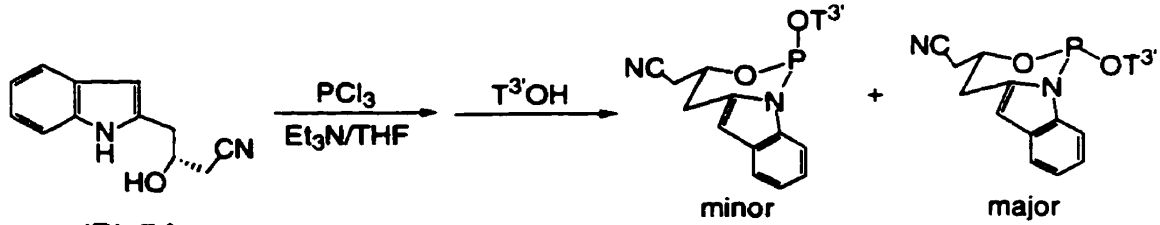
(S)-41 (S)-54

Using the same procedure as for the synthesis of (R)-3-hydroxy-4-(2-indolyl)-butyronitrile **54**, the reaction (S)-3-indol-2-yl-2-hydroxypropanyl p-toluenesulfonate **41** (1.62 g, 4.7 mmol) with sodium cyanide (0.5 g, 10.2 mmol) in a solution of LiCN (0.5 M in DMF, 20 ml) provided light yellow solid (S)-3-hydroxy-4-(2-indolyl)butyronitrile **54** (0.6 g) in 64% yield, m.p. 78 - 79 °C. $[\alpha]^{D}_{295}$ 7.67° (c 0.975, ethyl acetate).

The chiralty of (S)-**54** was 85% ee. After recrystallization in chloroform, (S)-**54** in 96% ee was obtained from the mother liquor. m.p. of the crystals 93 - 94 °C

^{1}H NMR (270 MHz, CDCl3): 8.42 (s, broad, 1H, NH), 7.0-7.5 (m, 4H, aromatic H), 6.30 (d, 1H, ^{4}J = 1.48 Hz, H-3-indole), 4.20 (m, 1H, CHO), 3.01 (m, 2H, CH_2), 2.47, 2.49 (m, 2H, CH_2CN). ^{13}C NMR (67.9 MHz, $CDCl_3$): 136.56, 133.58, 128.55, 121.94, 120.16, 120.06, 110.74, 102.12 (indole), 117.12 (CN), 67.55 (CH_2C), 35.10 (CHO), 25.28 (CH_2CN). MS (EI): 200 (59.6%, $M^{+\cdot}$), 130 (100%, M-$CNCH_2CH_2O$).

Rp-indole-oxazaphosphorine 56eq



(R)-54 Sp-56ax Rp-56eq

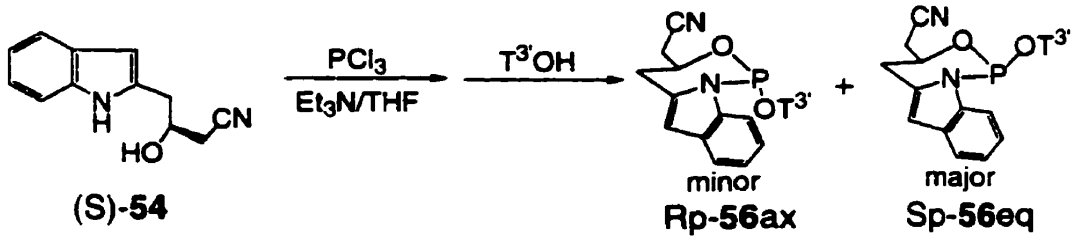
A dry 5-ml round-bottomed flask containing 1 ml of dry THF was flushed with argon and sealed with a septum, then 44 µl of PCl_3 (0.5 mmol) was introduced *via* a micro-syringe. The flask was cooled to -78 °C in a dry-ice/acetone bath, and a solution of (R)-3-hydroxy-4-(2-indolyl)butyronitrile **54** (100 mg, 0.5 mmol) in THF (1 ml) containing

triethylamine (0.3 ml, 2.2 mmol) was added *via* a syringe. The reaction mixture was stirred for 30 minutes at -78 ^{0}C, then warmed up to 0 ^{0}C for an hour. The flask was cooled to -78 ^{0}C again, and a solution of 5-O'-TBDMS-thymidine (178 mg, 0.5 mmol) in THF (0.5 ml) was added *via* a syringe. The reaction mixture was stirred at -78 ^{0}C for 30 minutes, then the cooling bath was removed and the solution was warmed up to room temperature. The triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 × 1 ml). The filtrate was concentrated and purified with TLC chromatography (CH_2Cl_2 : CH_3CN 2: 10) to afford white solid indole-oxazaphosphorine **56** (94 mg) in 32% yield, m.p. 85 - 86 ^{0}C.

Two diastereoisomers of indole-oxazaphosphorine Rp-**56**eq and Sp-**56**ax were obtained in a ratio of 30 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ Rp-**56**eq, 120.78 (96.8%), Sp-**56**ax 120.65 (3.2%). The following NMR spectra were assigned to the major one.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 (br s, 1H, NH), 7.39 (s, 1H, H-6), 7.57-7.17 (m, 4H, aromatic H), 6.42 (s, 1H, H-3-indole), 6.32 (dd, 1H, ^3J = 8.8, 5.0 Hz, H-1'), 4.80 (m, 1H, H-3'), 4.52 (m, 1H, CHOP), 4.02 (m, 1H, H-4'), 3.65, 3.23 (m, 2H, HH'-5'), 3.40, 3.25 (m, 2H, CH_2C), 2.91, 2.82 (m, 2H, CH_2CN), 2.38, 2.04 (m, 2H, HH'-2'), 1.87 (s, 3H, CH_3C-5), 0.85 (s, 9H, $SiC(CH_3)_3$), -0.004, -0.030 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.37 (C-4), 149.97 (C-2), 134.95, 133.35, 129.42, 129.41, 122.58, 121.69, 120.45, 115.64, 110.87, 110.25, 110.16, 104.23, 85.78 (d), 84.39, 74.47 (d), 69.44 (d), 62.62, 39.93 (d), 30.63 (d), 25.68, 25.36 9d), 18.07, 12.29, -5.69, -5.92. MS (FAB, NBA): 585 ($M+H^+$, 19.7%). HRMS (FAB, $M+H^+$): $C_{28}H_{38}N_4O_6PSi$, Calcd. 585.229827, Found 585.229850.

Sp-Indole-oxazaphosphorine 56eq



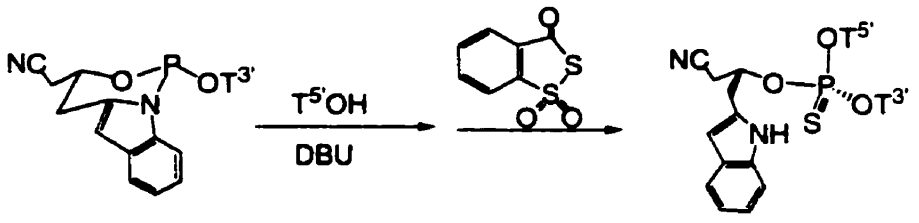
(S)-**54** Rp-**56ax** Sp-**56eq**

A dry 25-ml round-bottomed flask containing 10 ml of dry THF was flushed with argon and sealed with a septum. Then 206 μl of PCl_3 (2.36 mmol) was introduced *via* a micro-syringe. The flask was cooled to 0 ^{0}C in an ice-bath, and a solution of (S)-3-hydroxy-4-(2-indolyl)butyronitrile **54** (472 mg, 2.36 mmol) in THF (2 ml) containing triethylamine (1.2 ml, 8.6 mmol) was added *via* a syringe. The reaction mixture was stirred for 30 minutes at 0 ^{0}C, then warmed up to room temperature for half an hour. The flask was cooled to 0 ^{0}C again, and a solution of 5-O'-TBDMS-thymidine (800 mg, 2.3 mmol) in CH_2Cl_2 (4 ml) was added *via* a syringe. The reaction mixture was stirred at 0 ^{0}C for 30 minutes. The triethylammonium chloride was filtered off and washed with CH_2Cl_2 (2 × 5 ml). The filtrate was concentrated and purified by silica gel chromatography (CH_2Cl_2 : CH_3CN 1: 10) to afford white solid indole-oxazaphosphorine **56** (567 mg) in 41.2% yield, m. p. 93 - 94 ^{0}C.

Two diastereoisomers of indole-oxazaphosphorine Sp-**56**eq and Rp-**56**ax were obtained in a ratio of 6 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ Sp-**56**eq, 120.53 (85.3%), Rp-**56**ax, 120.72 (14.7%). The following NMR spectra were assigned for the major one.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.20 (br s, 1H, NH), 7.42 (s, 1H, H-6), 7.57-7.17 (m, 4H, aromatic H), 6.42 (s, 1H, H-3-indole), 6.34 (dd, 1H, ^3J = 8.8, 5.0 Hz, H-1'), 4.82 (m, 1H, H-3'), 4.47 (m, 1H, CHOP), 3.94 (m, 1H, H-4'), 3.76, 3.66 (m, 2H, HH'-5'), 3.40 - 3.24 (m, 2H, CH_2C), 2.91 (m, 2H, CH_2CN), 2.41, 2.01 (m, 2H, HH'-2'), 1.89 (s, 3H, CH_3C-5), 0.88 (s, 9H, $SiC(CH_3)_3$), 0.066, 0.072 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.66 (C-4), 150.41 (C-2), 138.11, 137.87, 135.14, 133.60, 129.67, 122.78, 121.86, 120.75, 116.13, 111.18, 110.51, 110.35, 104.41, 86.41, 86.35, 84.81, 75.49, 75.30, 70.71, 70.59, 63.15, 39.82, 30.88, 30.80, 25.96, 18.36, 12.55, -5.30, -5.41. MS (FAB, NBA): 557 (M-HCN, 44.5%).

Rp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl (R)-(1-cyano-3-indol-2-yl)isopropyl phosphorothioate 58



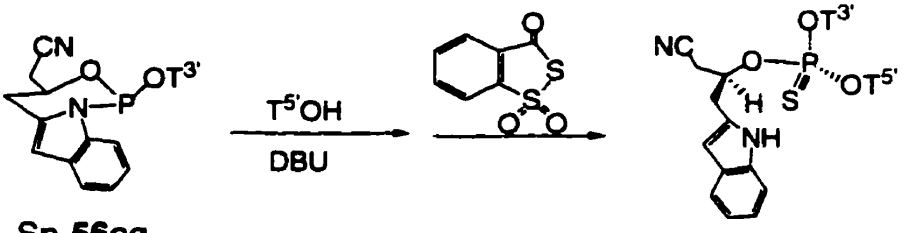
Rp-56eq **Rp-58**

To a solution of indole-oxazaphosphorine Rp-**56**eq/Sp-**56**ax (30:1) (80 mg, 0.137 mmol) in 0.5 ml dry THF was added 3'-O-TBDPS-thymidine ($T^{5'}OH$) (50 mg, 0.104 mmol) followed by DBU (40 µl, 0.268 mmol). This reaction mixture was shaken at room temperature for 10 minutes and passed though a short silica gel column to remove DBU. The column was eluted with CH_3CN. The solvent was evaporated to afford light yellow solid. This solid was redissolved in dry CH_2Cl_2 (2 ml), and Beaucage's reagent (35 mg, 0.175 mmol) was added. After 5 minutes, evaporation of the solvent followed by flash chromatography (CH_2Cl_2/CH_3COCH_3 5:1) afforded light yellow solid phosphorothioate triester Rp-**58** (84 mg) in 74% yield, m.p. 113 - 114 °C.

^{31}P NMR (202.3 MHz, $CDCl_3$): δ 66.55 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.21, 9.56, 9.14 (3 x s, br., 3H, NH-3-$T^{5'}$, NH-3-$T^{3'}$, NH-indole), 7.62 - 6.92 (m, 16H, aromatic H, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.35 (m, 1H, H-1'-$T^{5'}$), 6.28 (s, 1H, H-3-indole), 6.06 (dd, 1H, ^{3}J = 9.0, 5.5 Hz, H-1'-$T^{3'}$), 4.88 (m, 1H, CHOP), 4.82 (m, 2H, H-3'-$T^{3'}$), 4.32 (m, 1H, H-3'-$T^{5'}$), 4.06 (m, 1H, H-4'-$T^{5'}$), 3.93 (m, 1H, H-4'-$T^{3'}$), 3.88 (m, 1H, H-5'-$T^{5'}$), 3.71 (m, 2H, H-5'-$T^{3'}$, H'-5'-$T^{5'}$), 3.63 (m, 1H, H'-5'-$T^{3'}$), 3.21 (m, 2H, CCH_2), 2.61 (m, 2H, $CNCH_2$), 2.29, 1.95 (m, 2H, HH'-2'-$T^{5'}$), 1.90 (s, 3H, CH_3C-5-$T^{5'}$), 1.88 (s, 3H, CH_3C-5-$T^{3'}$), 1.82, 1.40 (m, 2H, HH'-2'-$T^{3'}$), 1.06 (s, 9H, $SiC(CH_3)_3$-$T^{5'}$), 0.88 (s, 9H, $SiC(CH_3)_3$-$T^{3'}$), 0.063, 0.059 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 164.13, 164.09 (C-4-$T^{3'}$, C-4-$T^{5'}$), 150.70, 150.35 (C-2-$T^{3'}$, C-2-$T^{5'}$), 135.82, 135.65, 135.54, 134.65, 132.75, 132.51, 131.97, 130.12 (d), 128.32, 127.93, 127.89, 121.45, 119.76, 119.56, 115.63, 111.22 (d), 110.80, 101.26, 85.24 (d), 84.94,

84.76 (d), 84.44, 80.32, 74.02 (d), 72.55, 67.22 (d), 63.02, 39.89, 38.16, 33.18 (d), 26.67, 25.75, 23.45, 18.81, 18.12, 12.41 (d), -5.56, -5.62.

Sp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl (S)-(1-cyano-3-indol-2-yl)isopropyl phosphorothioate 58



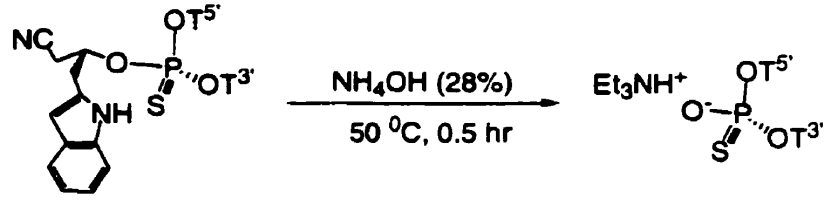
Sp-**56**eq Sp-**58**

To a solution of indole-oxazaphosphorine Sp-**56**eq/Rp-**56**ax (6:1) (307 mg, 0.526 mmol) in 10 ml dry THF was added 3'-O-TBDPS-thymidine ($T^{5'}OH$) (253 mg, 0.526 mmol) followed by DBU (157 µl, 1.05 mmol). The reaction mixture was stirred at room temperature for 5 minutes and passed though a short silica gel column to filter off DBU. The column was eluted with CH_3CN. The solvent was evaporated to afford light yellow solid. This solid was redissolved in dry CH_2Cl_2 (5 ml), and Beaucage's reagent (150 mg, 0.75 mmol) was added. After 10 minutes, evaporation of the solvent followed by flash chromatography (CH_2Cl_2/CH_3COCH_3 5:1) afforded light yellow solid phosphorothioate triester Sp-**58** (388.4 mg) in 68% yield, m.p. 116 - 117 ^{0}C.

^{31}P NMR (202.3 MHz, $CDCl_3$): δ 66.31 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.16, 9.13, 8.60 (3 x s, 3H, NH-3-$T^{5'}$, NH-3-$T^{3'}$, NH-indole), 7.63 - 7.05 (m, 16H, aromatic H, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.33 (s, 1H, H-3-indole), 6.27 (m, 1H, H-1'-$T^{5'}$), 6.17 (dd, 1H, ^3J = 9.0, 5.0 Hz, H-1'-$T^{3'}$), 4.91 (m, 2H, H-3'-$T^{3'}$, CHOP), 4.30 (m, 1H, H-3'-$T^{5'}$), 4.10 (m, 1H, H-4'-$T^{5'}$), 3.88 (m, 1H, H-4'-$T^{3'}$), 3.79 (m, 2H, H-4'-$T^{3'}$, H-5'-$T^{5'}$), 3.54, 3.45 (m, 2H, HH'-5'-$T^{3'}$), 3.19 (m, 2H, CCH_2), 2.65 (m, 2H, $CNCH_2$), 2.28 (m, 2H, H-2'-$T^{3'}$, H-2'-$T^{5'}$), 2.01 (m, 1H, H'-2'-$T^{5'}$), 1.89 (s, 3H, CH_3C-5-$T^{5'}$), 1.86 (s, 3H, CH_3C-5-$T^{3'}$), 1.87 (m, 1H, H'-2'-$T^{3'}$), 1.06 (s, 9H, $SiC(CH_3)_3$-$T^{5'}$), 0.88 (s, 9H,

$SiC(CH_3)_3$-$T^{3'}$), 0.068, 0.060 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.82, 163.17 (C-4-$T^{3'}$. C-4-$T^{5'}$), 150.35, 150.27(C-2-$T^{3'}$, C-2-$T^{5'}$), 136.33, 136.10, 135.79, 134.85, 132.96, 132.81, 131.61, 130.30, 128.33, 128.13, 128.06, 122.20, 120.24, 115.85, 111.36, 111.24, 110.94, 102.61, 86.32, 85.49, 85.40, 85.13, 85.01, 84.60, 80.75, 80.68, 77.29, 74.23, 74.15, 73.06, 67.86, 63.16, 39.92, 38.92 (d), 33.57 (d), 31.00, 26.90, 25.96, 23.96, 23.65, 19.06, 18.34, 12.58, 12.52, -5.33, -5.41. MS (FAB, NBA): 1097 (11.1%, M+H^+), 759 (5.4%), 377 (28.1%), 339 (51.7%), 182 (100%).

Sp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl phosphorothioate 59



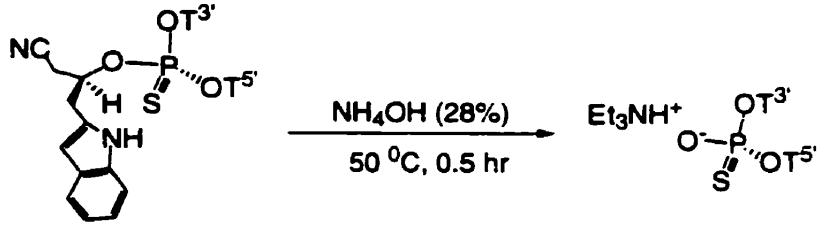
Rp-**58** Sp-**59**

To a solution of phosphorothioate triester Rp-**58** (60 mg, 55.8 μmol) in 1 ml of methanol was added 20 ml of aqueous ammonia (28%). The solution was stirred at 50 °C for half an hour, neutralized with HCl (6N) and extracted with ethyl acetate (3 × 10 ml). The combined extracts were dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was purified by flash chromatography (acetone/triethylamine 10:1) to give white solid Sp-**59** (32 mg) which existed as a triethylammonium salt in 58% yield, m.p. 97 - 98 °C.

^{31}P NMR (202.3 MHz, CD_3OD): δ 58.96 ppm. ^1H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, 1H, J = 1.0 Hz, H-6-$T^{5'}$), 7.56, 7.31 (m, 10H, $C_6H_5SiC_6H_5$), 7.48 (d, 1H, J = 1.0 Hz, H-6-$T^{3'}$), 6.39 (dd, 1H, J = 9.2, 5.0 Hz, H-1'-$T^{5'}$), 6.05 (dd, 1H, J = 8.8, 5.0 Hz, H-1'-$T^{3'}$), 4.88 (m, 1H, H-3'-$T^{3'}$), 4.47 (m, 1H, H-3'-$T^{5'}$), 3.98 (m, 1H, H-4'-$T^{3'}$), 3.95 (m, 1H, H-4'-$T^{5'}$), 3.74, 3.50 (m, 2H, HH'-5'-$T^{3'}$), 3.67 (m, 2H, HH'-5'-$T^{5'}$),

2.86, 1.10 (q, t, $N(CH_2CH_3)_3$), 2.28 (m, 1H, H-2'-$T^{3'}$), 2.00 (m, 3H, H'-2'-$T^{3'}$, HH'-2'-$T^{5'}$), 1.83 (d, 3H, J = 1.0 Hz , CH_3C-5-$T^{5'}$), 1.76 (d, 3H, J = 1,0 Hz, CH_3C-5-$T^{3'}$), 0.98 (s, 9H, $SiC(CH_3)_3$-$T^{5'}$), 0.80 (s, 9H, $SiC(CH_3)_3$-$T^{3'}$), 0.009, 0.003 (ss, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.38, 166.28 (C-4-$T^{3'}$. C-4-$T^{5'}$), 152.48, 151.95 (C-2-$T^{3'}$, C-2-$T^{5'}$), 138.04, 137.26, 136.82, 136.80, 134.35, 134.25, 131.18, 131.08, 129.02, 128.94, 112.22, 111.09, 88.03, 87.95, 87.61 (d), 86.34, 86.16, 77.98 (d), 76.30, 66.46 (d), 64.81, 41.36, 40.58 (d), 27.29, 26.41, 19.68, 19.16, 12.60 (d), -5.22, -5.28. MS (FAB, NBA): 937 ($M+Na^+$)

Rp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl phosphorothioate 59



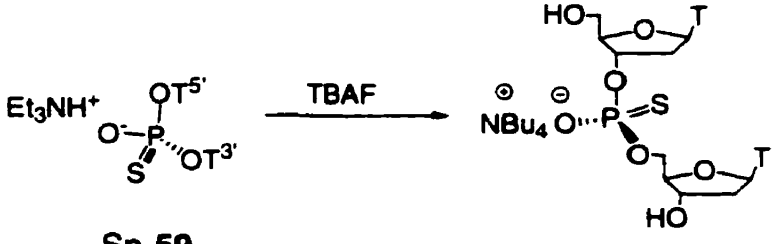
Sp-**58** Rp-**59**

Using the same procedure as for the synthesis of Sp-**59**, a solution of phosphorothioate triester Sp-**58** (0.2248 g, 0.205 mmol) in 20 ml of aqueous ammonia (28%) was stirred at 50 °C for half an hour. After purification by flash chromatography (acetone/triethylamine 10:1) gave white solid Rp-**59** (0.2 g) in quantitative yield.

^{31}P NMR (202.3 MHz, CD_3OD): δ 59.07 ppm. ^{1}H NMR (500 MHz, CD_3OD): δ 7.69 (d, 1H, J = 1.0 Hz, H-6-$T^{5'}$), 7.49, 7.24 (m, 10H, $C_6H_5SiC_6H_5$), 7.44 (d, 1H, J = 1.0 Hz, H-6-$T^{3'}$), 6.32 (dd, 1H, J = 9.0, 5.5 Hz, H-1'-$T^{5'}$), 6.02 (dd, 1H, ^{3}J = 8.5, 5.5 Hz, H-1'-$T^{3'}$), 4.83 (m, 1H, H-3'-$T^{3'}$), 4.40 (m, 1H, H-3'-$T^{5'}$), 4.05 (m, 1H, H-4'-$T^{3'}$), 3.92 (m, 1H, H-4'-$T^{5'}$), 3.72 (m, 2H, H-5'-$T^{3'}$, H-5'-$T^{5'}$), 3.45 (m, 1H, H'-5'-$T^{5'}$), 2.84, 1.04 (q, t, $N(CH_2CH_3)_3$), 2.07 (m, 2H, H-2'-$T^{3'}$, H-2'-$T^{5'}$), 1.92 (m, 1H, H'-2'-$T^{5'}$), 1.80 (m, 1H, H'-2'-$T^{3'}$), 1.76 (br, H_2O, CH_3C-5-$T^{5'}$), 1.71 (d, 3H, J = 1,0 Hz, CH_3C-5-$T^{3'}$), 0.91 (s, 9H, $SiC(CH_3)_3$-$T^{5'}$), 0.75 (s, 9H, $SiC(CH_3)_3$-$T^{3'}$), -0.041, -0.047 (ss, 6H,

$Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.36, 166.21 (C-4-$T^{3'}$, C-4-$T^{5'}$), 152.44, 151.96 (C-2-$T^{3'}$, C-2-$T^{5'}$), 138.03, 137.11, 136.86, 136.81, 134.36, 134.26, 131.19, 131.13, 129.07, 129.00, 112.13, 111.26, 88.11, 88.06, 88.04, 88.03, 86.29, 88.24, 78.53, 78.50, 76.20, 66.47, 66.43, 64.90, 47.45, 41.57, 40.43, 40.39, 27.39, 26.51, 22.08, 19.74, 19.04, 12.72, 12.67, 9.68, -5.08, -5.16.

Sp-Tetrabutylammonium thymid-3'-yl thymid-5'-yl phosphorothioate 60



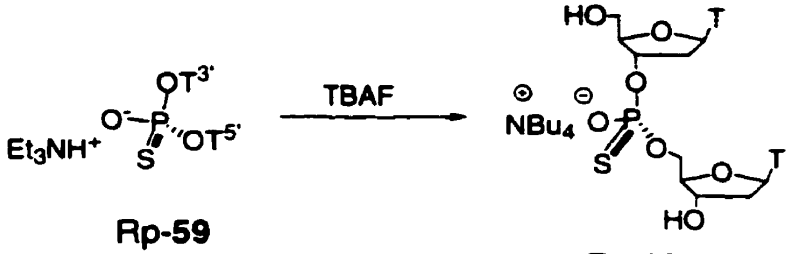
Sp-**59** Sp-**60**

A solution of TBAF (1.0 M in DMF, 4 ml) containing dimer Sp-**59** (20 mg, 17.5 μmol) was stirred at room temperature for one hour. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (acetone/triethylamine 1:1) to give a sticky solid dimer Sp-**60** (15 mg) which existed as a tetrabutylammonium salt in 95% yield.

^{31}P NMR (202.3 MHz): δ 58.96 ppm (CD_3OD), 55.45 ppm (D_2O). ^{1}H NMR (500 MHz, CD_3OD): δ 7.80 (d, 1H, J = 1.0 Hz, H-6-$T^{5'}$), 7.78 (d, 1H, J = 1.5 Hz, H-6-$T^{3'}$), 6.29 (dd, 1H, J = 8.0, 6.0 Hz, H-1'-$T^{5'}$), 6.22 (dd, 1H, J = 8.0, 6.0 Hz, H-1'-$T^{3'}$), 4.98 (m, 1H, H-3'-$T^{3'}$), 4.45 (m, 1H, H-3'-$T^{5'}$), 4.12 (m, 1H, H-4'-$T^{3'}$), 4.09, 3.98 (m, 2H, HH'-5'-$T^{5'}$), 3.98 (m, 1H, H-4'-$T^{5'}$), 3.74 (m, 2H, HH'-5'-$T^{3'}$), 3.17, 1.59, 1.35, 0.94 ($N(CH_2CH_2CH_2CH_3)_4$), 2.40 (m, 1H, H-2'-$T^{3'}$), 2.20 (m, 2H, H'-2'-$T^{3'}$, H-2'-$T^{5'}$), 2.12 (m, 1H, H'-2'-$T^{5'}$), 1.89 (d, 3H, J = 1.0 Hz, CH_3C-5-$T^{5'}$), 1.80 (d, 3H, J = 1,0 Hz, CH_3C-5-$T^{3'}$). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.45, 166.34 (C-4-$T^{3'}$, C-4-$T^{5'}$), 152.40, 152.24 (C-2-$T^{3'}$, C-2-$T^{5'}$), 138.08, 138.06, 112.02, 111.53, 87.61 (d), 87.31

(d), 86.16 (d), 77.06 (d), 72.87, 66.69 (d),62.79, 59.39 (t), 40.79, 40.01 (d), 24.69, 20.61, 13.85, 12.60, 12.37.

Rp-Tetrabutylammonium thymid-3'-yl thymid-5'-yl phosphorothioate 60



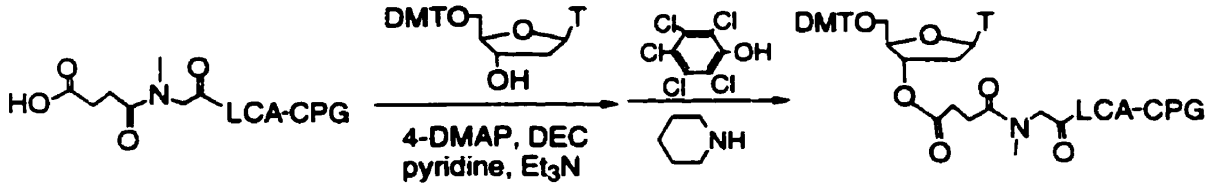
Rp-59 Rp-60

Using the same procedure as for the synthesis of Sp-**60**, desilylation of Rp-**59** (80.4 mg, 0.079 mmol) with TBAF provided sticky solid dimer Rp-**60** (50 mg) in 78% yield.

Two diastereomers Rp-**60** and Sp-**60** were observed from its ^{31}P NMR in a ratio of 21:1. ^{31}P NMR (202.3 MHz, CD_3OD): δ Rp-**60**, 58.92 ppm (95.4%), Sp-**60**, 58.99 ppm (4.6%).

^{1}H NMR (500 MHz, CD_3OD): δ 7.85 (d, 1H, J = 1.0 Hz, H-6-$T^{5'}$), 7.80 (d, 1H, J = 1.0 Hz, H-6-$T^{3'}$), 6.29 (dd, 1H, J = 8.0, 6.0 Hz, H-1'-$T^{5'}$), 6.22 (dd, 1H, ^{3}J = 8.2, 5.5 Hz, H-1'-$T^{3'}$), 5.00 (m, 1H, H-3'-$T^{3'}$), 4.45 (m, 1H, H-3'-$T^{5'}$), 4.15 (m, 1H, H-4'-$T^{3'}$), 4.06 (m, 2H, HH'-5'-$T^{3'}$), 3.98 (m, 1H, H-4'-$T^{5'}$), 3.77 (m, 2H, HH'-5'-$T^{5'}$), 3.17, 1.59, 1.35, 0.95 ($N(CH_2CH_2CH_2CH_3)_4$), 2.40 (m, 1H, H-2'-$T^{5'}$), 2.20 (m, 2H, H-2'-$T^{3'}$, H'-2'-$T^{5'}$), 2.14 (m, 1H, H'-2'-$T^{3'}$), 1.91 (d, 3H, J = 1.o Hz, CH_3C-5-$T^{5'}$), 1.81 (d, 3H, J = 1,0 Hz, CH_3C-5-$T^{3'}$). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.43, 166.28 (C-4-$T^{3'}$, C-4-$T^{5'}$), 152.39, 152.21 (C-2-$T^{3'}$, C-2-$T^{5'}$), 138.12, 138.03, 112.00, 111.48, 87.85, 87.80, 87.49, 87.42, 86.20, 86.08, 77.49, 77.45, 72.94, 66.35, 66.30, 62.83, 59.44, 59.42, 59.39, 40.87, 39.88, 39.84, 24.70, 20.62, 13.87, 12.61, 12.40.

The loading of 5'-O-DMT-thymidine on CPG 62a



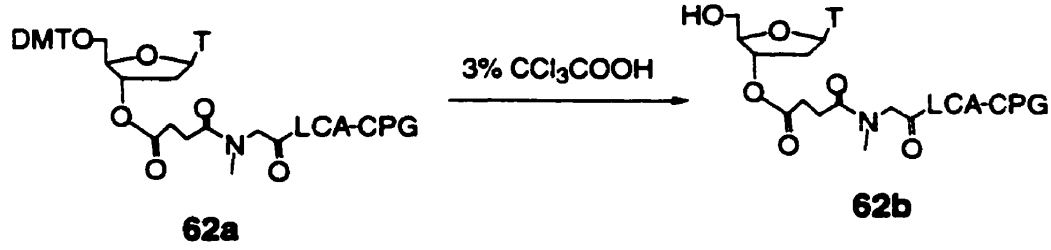
61 **62a**

To a dry 6 ml-hypovials was added 5'-O-DMT-thymidine (109 mg, 0.2 mmol), CPG with sarcosinyl-succinonyl linker (1.0 g), 4-DMAP (12 mg, 0.1 mmol), triethylamine (80 µl), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC) (384 mg, 2.0 mmol) and anhydrous pyridine (5 ml). The mixture was shaken at room temperature for 24 hours. Pentachlorophenol (134 mg, 0.5 mmol) was added, and the mixture was shaken for an additional period of 16 hours. The CPG was filtered off and washed successively with pyridine, CH_2Cl_2, and ether. Then the CPG was treated with reagent grade piperidine (5 ml), and the slurry was shaken for 10 minutes. The resulting CPG was filtered off, washed successively with CH_2Cl_2 and ether, and dried under vacuum.

The next was a capping step. The dry CPG was mixed with equal parts of two solutions of 0.5 M acetic anhydride in THF and 0.5 M 4-DMAP/2,4,6-trimethylpridine in THF (4 ml each). The slurry was shaken for 2 hours, then washed successively with pyridine, CH_2Cl_2, THF and ether. Drying under vacuum afforded the immobilized thymidine **62a** (0.89 g).

The loading amount was measured by Trityl Analysis. 6.0 mg of **62a** was treated with 10 ml of 5% trichloroacetic acid in 1,2-dichloroethane. The UV absorbance of the solution was 1.7283 at 504 nm. The loading amount was therefore 37.9 µmol/g according to the calculation $(A \cdot V \cdot 10^3)/(76W)$. (A: the UV absorbance at 504 nm; V: volume of the solution; W: the weight of CPG.)

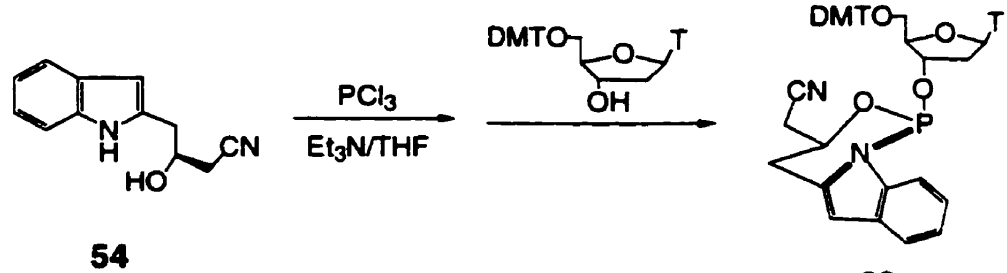
The immobilized thymidine 62b



62a **62b**

The immobilized thymidine **62a** (0.5 g) was placed in a sintered glass funnel and washed with 3% trichloroacetic acid until no red color was observed. The resulting CPG was dried under vacuum to afford the immobilized thymidine **62b**.

Sp-Indole-oxazaphosphorine 63



54 **63**

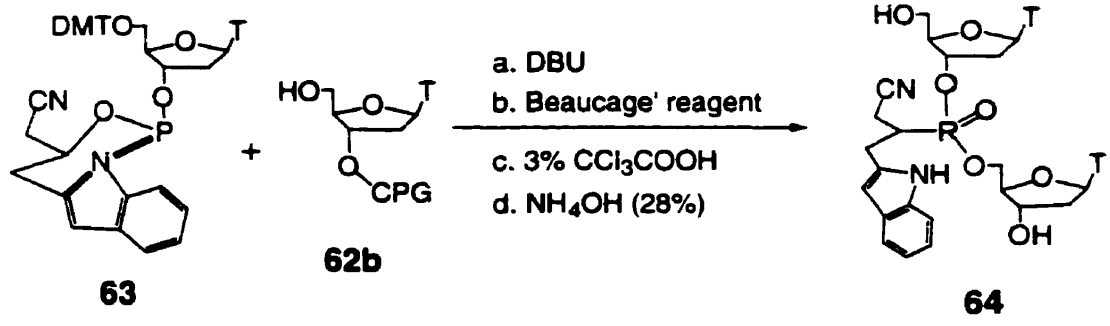
Using the same procedure as for the synthesis of indole-oxazaphosphorine **56**, the reaction of (S)-**54** (3.0 g, 15 mmol) with PCl_3 (1.3 ml, 15 mmol) and 5'-O-DMT-thymidine (8.2 g, 15 mmol) afforded light yellow solid indole-oxazaphosphorine Sp-**63** (5.43 g) in 47% yield, m.p. 112 - 113 ^{0}C.

Two diastereoisomers of indole-oxazaphosphorine **63** were obtained in a ratio of 13 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 121.58 ppm (93%), 122.17 ppm (7.0%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.93 (br s, 1H, NH), 7.55 (s, 1H, H-6), 7.24, 6.83 (m, 17H, aromatic H), 6.40 (m, 2H, H-3-indole, H-1'), 4.95 (m, 1H, H-3'), 4.41 (m, 1H, CHOP), 4.04 (m, 1H, H-4'), 3.80 (d, 6H, 2 × OCH_3), 3.50, 3.31 (m, 2H, HH'-5'), 3.10 (m, 2H, CH_2-Indole), 2.72 (m, 2H, CH_2CN), 2.49, 2.24 (m, 2H, HH'-2'),

1.41 (s, 3H, CH_3C-5). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.44 (C-4), 158.62 (C-2), 150.20, 143.92, 137.74, 137.61, 135.22, 134.98, 134.93, 133.58, 129.96, 128.00, 127.85, 127.09, 122.55, 121.63, 120.44, 115.76, 113.13 (d), 111.33, 110.30 (d), 104.17, 86.89, 85.18 (d), 84.49, 75.07 (d), 70.20 (d), 62.75, 55.15, 39.45 (d), 30.75 (d), 29.52, 25.14 (d), 11.56. MS (FAB, NBA): 795 (M+Na$^+$, 4.6%), 773 (M+H$^+$, 1.0%), 772 (1.8%).

Alkylphosphonate 64

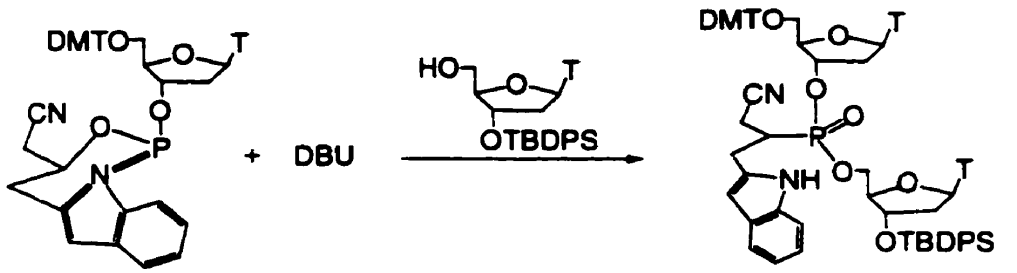


63 **62b** **64**

To the sintered glass funnel was added solid support **62b** (27 mg, 1 μmol) and a solution of indole-oxazaphosphorine Sp-**63** in acetonitrile (0.2 ml, 0.1 M), then 30 μl of DBU (0.2 mmol) was added by a syringe. After five minutes, the solid support was washed with acetonitrile (3 x 2 ml) and treated with Beaucage's reagent (0.1 ml, 0.1 M in THF). After detritylation with 3% trichloroacetic acid in 1,2-dichloroethane, the solid support was cleaved with NH_4OH (28%) at 50 °C for two hours. The solution was evaporated to dryness with a phase-drier, the residue was dissolved in HPLC-grade water (1 ml) and filtered to give a solution of alkylphosphonate **64**. The HPLC analysis see Scheme 26.

MS (FAB) m/z: 735 (M+Na$^+$, 4.52%), 713 (M+H$^+$, 100.0%), 714 (42.95%), 715 (9.40%).

Alkylphosphonate 66



63 **66**

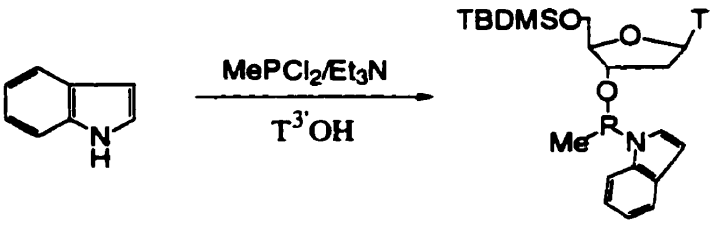
To a solution of Sp-**63**/Rp-**63** (12:1) (200 mg, 0.26 mmol) in dry THF (10 ml) was added 5 eq. of DBU (194 µl). After 5 minutes, 1 eq. of 3'-O-TBDPS-thymidine (124 mg) was added. The mixture was stirred for 5 minutes. The solvent was evaporated, and the crude product was purified by flash chromatography (EtOAc) to give 140 mg of alkylphosphonate **66** as light yellow solid in 43% yield, m.p. 127-128 ^{0}C.

Four peaks of ^{31}P NMR in alkylphosphonate **66** were observed. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 29.88 ppm (14.2%), 29.66 ppm (1.7%), 28.97 ppm (21.0%), 28.79 ppm (63.1%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.52 (s, 1H, NH-3-$T^{5'}$), 9.44 (s, 1H, NH-3-$T^{3'}$), 9.10 (s, 1H, NH), 7.62 - 6.77 (m, 27H, aromatic H), 7.48 (s, 1H, H-6-$T^{5'}$), 7.13 (s, 1H, H-6-$T^{3'}$), 6.30 (m, 2H, H-1'-$T^{3'}$, NCCH), 6.13 (m, 1H, H-1'-$T^{5'}$), 5.16 (m, 1H, H-3'-$T^{3'}$), 4.29 (m, 1H, H-3'-$T^{5'}$), 4.10 (m, 1H, H-4'-$T^{5'}$), 4.06 (m, 1H, H-4'-$T^{3'}$), 3.88, 3.82 (m, 2H, HH'-5'-$T^{3'}$), 3.73, 3.72 (d, 6H, 2 x CH_3O), 3.43, 3.28 (m, 2H, HH'-5'-$T^{5'}$), 3.17 (m, 2H, CCH_2), 2.90 (m, 1H, PCH), 2.50 (m, 1H, H-2'-$T^{3'}$), 2.36 (m, 3H, H'-2'-$T^{3'}$, $CNCH_2$), 2.24, 2.06 (m, HH'-2'-$T^{5'}$), 1.77 (s, 3H, CH_3C-5-$T^{5'}$), 1.43 (s, 3H, CH_3C-5-$T^{3'}$), 1.06 (s, 9H, $SiC(CH_3)_3$). HRMS (FAB, M+H$^+$): $C_{69}H_{74}N_6O_{13}PSi$, Calcd. 1253.482079, Found 1253.482500.

4.3. Experiments for Chapter III

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl indolmethylphosphorine 87

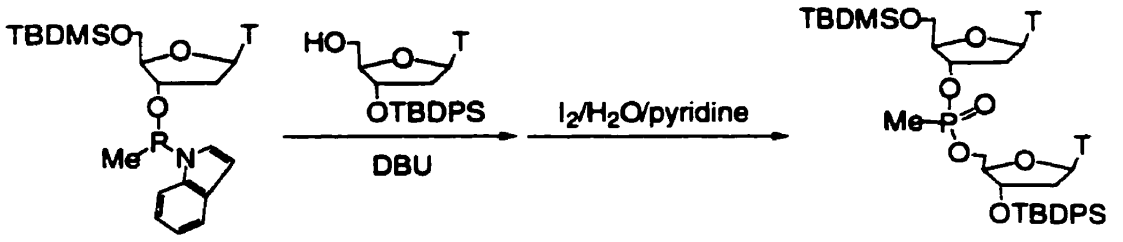


87

Route A: To a solution of indole (50 mg, 0.43 mmol) and 132 µl of triethylamine (0.94 mmol) in 2 ml of dry dichloromethane was added 42.5 µl of dichloromethyl-phosphorine (0.43 mmol) at 0 °C. The solution was shaken for ten minutes, then 152 mg of 5'-O-TBDMS-thymidine (0.43 mmol) in 0.5 ml of dry dichloromethane was added. The reaction was complete after a few minutes, and two diastereoisomers of **87** were quantitatively formed as established by ^{31}P NMR (128.58 ppm, 129.50 ppm, 1:1). This solution was used as such for further reactions.

Route B: To a solution of indole (100 mg, 2 eq.) and 132 µl of triethylamine (2.2 eq.) in 2 ml of dry dichloromethane was added 42.5 µl of dichloromethylphosphorine (1 eq.) at 0 °C. Then 5'-O-TBDMS-thymidine (152 mg, 1eq.) in 0.5 ml of dry dichloromethane was added. The mixture was kept at 50 °C for 3 hours to provide **87**. This solution was used as such for further reactions. The excess indole did not interfere with the next reactions.

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl methylphosphonates 89



87 **89**

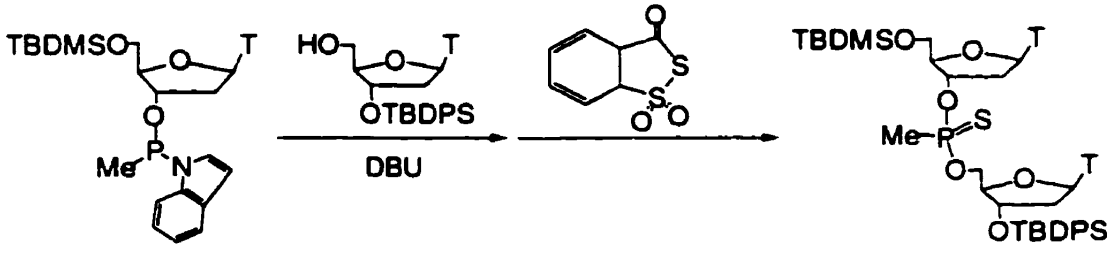
To a solution of **87** (from route A, 0.43 mmol) was added a solution of 3'-O-TBDPS-thymidine (220 mg, 0.46 mmol) in dichloromethane (1 ml) followed by 129 µl of DBU (0.86 mmol) *via* a syringe. The mixture was shaken and kept at 50 ^{0}C for 30 minutes. The solution was treated with 1 ml of 0.1M of iodine in THF-pyridine-H_2O (4:3:3 v/v) and shaken for 5 minutes. Purification with flash chromatography (ethyl acetate) afforded two isomers of methylphosphonates **89**, the first one as colorless sticky solid (Rp-**89**) (172 mg, R_f = 0.42) in 45% yield, and the second one as light yellow solid (Sp-**89**) (176 mg, R_f = 0.25) in 46% yield.

Rp-**89**, ^{31}P NMR (109.3 MHz, $CDCl_3$): δ 33.29 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.73 (br, s, 2H, NH-$T^{3'}$, NH-$T^{5'}$), 7.31-7.62 (m, 12H, $C_6H_5SiC_6H_5$, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.37 (m, 1H, H-1'-$T^{5'}$), 6.27 (dd, J = 9.0 Hz, 5.0 Hz, 1H, H-1'-$T^{3'}$), 4.95 (m, 1H, H-3'-$T^{3'}$), 4.30 (m, 1H, H-3'-$T^{5'}$), 4.12 (m, 1H, H-4'-$T^{3'}$), 4.08 (m, 1H, H-4'-$T^{5'}$), 3.88, 3.57 (m, 2H, H-5', H-5''- $T^{5'}$), 3.82 (m, 2H, H-5', H-5''-$T^{3'}$), 2.33, 1.81 (m, 2H, H-2', H-2''-$T^{5'}$), 2.30, 1.98 (m, 2H, H-2', H-2''-$T^{3'}$), 1.90 (s, 3H, CH_3C-5-$T^{5'}$), 1.86 (s, 3H, CH_3C-5-$T^{3'}$), 1.37 (d, $^{3}J_{P-H}$ = 17.5 Hz, 3H, CH_3P), 1.06 (s, 9H, C(CH_3)3-$T^{5'}$), 0.88 (s, 9H, C(CH_3)3-$T^{3'}$), 0.069 (ss, 6H, CH_3SiCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 179.63, 164.13, 150.61, 150.55, 135.77, 135.75, 135.48, 134.89, 133.05, 132.78, 130.28, 130.25, 128.07, 111.34, 111.31, 86.00, 85.39, 85.30, 84.58, 73.09, 65.00, 64.91, 63.22, 42.92, 40.48, 26.89, 25.95, 19.06, 18.33, 12.58, 12.42, 10.60, -5.33, -5.44. MS (FAB, NBA): 897 ($M+H^+$).

Sp-**89**, m.p. 94-95^{0}C. ^{31}P NMR (109.3 MHz, $CDCl_3$): δ 34.17 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.66 (br, s, 1H, NH-$T^{3'}$), 8.59 (br, s, 1H, NH-$T^{5'}$), 7.22-7.65 (m, 12H, $C_6H_5SiC_6H_5$, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.39 (m, 1H, H-1'-$T^{5'}$), 6.30 (dd, ^{3}J = 9.5 Hz, 5.0

Hz, 1H, H-1'-$T^{3'}$), 4.96 (m, 1H, H-3'-$T^{3'}$), 4.28 (m, 1H, H-3'-$T^{5'}$), 4.06-4.14 (m, 2H, H-4'-T^{3}, H-4'-$T^{5'}$), 3.76-3.90 (m, 4H, H-5', H-5''- $T^{5'}$, H-5', H-5''-$T^{3'}$), 2.40, 2.08 (m, 2H, H-2', H-2''-$T^{3'}$), 2.35, 1.84 (m, 2H, H-2', H-2''-$T^{5'}$), 1.91 (s, 3H, CH_3C-5-$T^{5'}$), 1.88 (s, 3H, CH_3C-5-$T^{3'}$), 1.42 (d, $^3J_{P-H}$ = 17.5 Hz, 3H, CH_3P), 1.08 (s, 9H, $C(CH_3)3$-$T^{5'}$), 0.90 (s, 9H, $C(CH_3)3$-$T^{3'}$), 0.10 (ss, 6H, CH_3SiCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.97, 163.91, 150.60, 150.47, 135.77, 135.73, 134.89, 132.92, 132.76, 130.34, 130.28, 128.09, 128.06, 111.46, 111.31, 86.06, 85.31, 85.15, 84.69, 72.81, 65.02, 64.93, 63.24, 40.39, 39.65, 26.89, 25.97, 19.06, 18.34, 12.58, 12.52, 10.59, -5.35, -5.40.

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (3'-O-*tert*-butyldiphenylsilyl)-thymid-5'-yl methylthiophosphonates 90



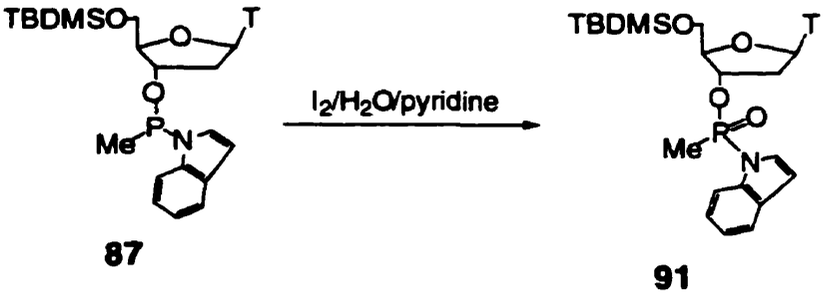
87 **90**

To a solution of **87** (from Route A, 0.25 mmol) was added a solution of 3'-O-TBDPS-thymidine (120 mg, 0.25 mmol) dichloromethane (1 ml) followed by 75 µl of DBU (0.5 mmol). The mixture was shaken and kept at 50 °C for 30 minutes. The solution was treated with Beaucage's reagent (60 mg, 0.3 mmol). The solvent was evaporated and the residue was purified by flash chromatography (hexanes/ethyl acetate 2:1) to give two diastereomers of methylthiophosphonates **90**, the first one as a colorless oil **90a** (58.2 mg, R_f = 0.72) in 26% yield, and the second one **90b** (10.3 mg, R_f = 0.41) in 5% yield. The two diastereomers of **90** was approximately in same ratio in its ^{31}P NMR (unseparated mixture).

90a, ^{31}P NMR (109.3 MHz, $CDCl_3$): δ 100.12 ppm. ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.59 (br, s, 2H, NH-$T^{3'}$, NH-$T^{5'}$), 7.62-7.20 (m, 12H, $C_6H_5SiC_6H_5$, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.39 (dd, ^{3}J = 7.7 Hz, 5.7 Hz, 1H, H-1'-$T^{5'}$), 6.30 (dd, ^{3}J = 9.5 Hz, 5.0 Hz, 1H, H-1'-$T^{3'}$), 5.15 (dd, ^{3}J = 10.9 Hz, 5.2 Hz, 1H, H-3'-$T^{3'}$), 4.28 (m, 1H, H-3'-$T^{5'}$), 4.06 (m, 1H, H-4'-$T^{3'}$), 4.00 (m, 1H, H-4'-$T^{5'}$), 3.86, 3.65 (m, 2H, HH'-5'- $T^{5'}$), 3.79 (m, 2H, HH'-5'-$T^{3'}$), 2.32, 2.05 (m, 2H, HH'-2'-$T^{5'}$), 1.75 (m, 2H, HH'-2'-$T^{3'}$), 1.91 (s, 3H, CH_3C-5-$T^{5'}$), 1.90 (s, 3H, CH_3C-5-$T^{3'}$), 1.72 (d, $^{3}J_{P-H}$ = 17.5 Hz, 3H, CH_3P), 1.07 (s, 9H, C(CH_3)3-$T^{5'}$), 0.91 (s, 9H, C(CH_3)3-$T^{3'}$), 0.11 (ss, 6H, CH_3SiCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.62, 163.54, 150.41, 150.24, 135.81, 135.74, 135.08 (d), 132.92 (d), 130.32, 128.11 (d), 111.46, 111.38, 86.21, 85.37, 85.12, 84.77, 72.97, 65.38, 63.18, 40.55, 39.56, 26.92, 25.98, 19.07, 18.40, 12.58, -5.31. MS (FAB, NBA): 913 ($M+H^+$).

90b, ^{31}P NMR (109.3 MHz, $CDCl_3$): δ 98.67 ppm. ^{1}H NMR (270 MHz, $CDCl_3$): 9.52 (br, s, 2H, NH-$T^{3'}$, NH-$T^{5'}$), 7.61-7.20 (m, 12H, $C_6H_5SiC_6H_5$, H-6-$T^{3'}$, H-6-$T^{5'}$), 6.32 (m, 2H, H-1'-$T^{5'}$, H-1'-$T^{3'}$), 5.13 (dd, ^{3}J = 11.6 Hz, 5.4 Hz, 1H, H-3'-$T^{3'}$), 4.43 (m, 1H, H-3'-$T^{5'}$), 4.27 (m, 1H, H-4'-T^{3}), 4.10 (m, 2H, H-4'-$T^{5'}$, H-5'-$T^{3'}$), 3.95, 3.42 (m, 2H, HH'-5'-$T^{3'}$), 3.84 (m, 1H, H'-5'-$T^{5'}$), 2.35, 1.98 (m, 2H, HH'-2'-$T^{3'}$), 2.15, 1.90 (m, 2H, HH'-2'-$T^{5'}$), 1.91, 1.87 (2 × s, 6H, CH_3C-5-$T^{5'}$, CH_3C-5-$T^{3'}$), 1.70 (d, $^{3}J_{P-H}$ = 17.5 Hz, 3H, CH_3P), 1.05 (s, 9H, C(CH_3)3-$T^{5'}$), 0.89 (s, 9H, C(CH_3)3-$T^{3'}$), 0.10 (ss, 6H, CH_3SiCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 164.10, 164.04, 150.63, 150.47, 135.81, 135.78, 135.40, 134.93, 133.08, 132.78, 130.29 (d), 130.17 (d), 128.11 (d), 127.95, 111.42, 111.31, 111.03, 87.80, 86.73, 86.36, 86.28, 85.69, 85.57, 85.45, 84.67, 73.39, 73.12, 65.36 (d), 63.29, 62.08, 60.47, 40.69, 40.39, 39.29 (d), 26.96 (d), 26.00, 19.08 (d), 18.39, 12.61 (d), -5.29, -5.32.

Indole derivative 91



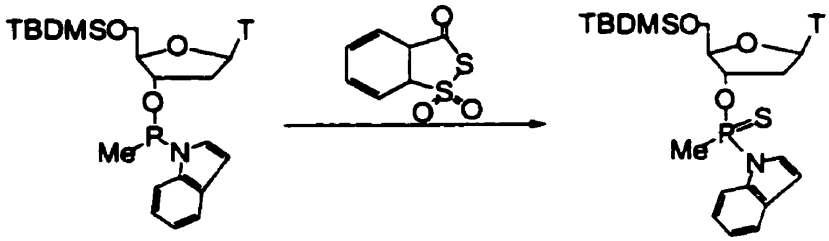
87 **91**

A solution of **87** (from Route A, 0.25 mmol) was treated with 1ml of 0.1M of iodine in THF-pyridine-H_2O (4:3:3 v/v) for 5 minutes. The mixture was purified by flash chromatography (ethyl acetate) to give two diastereomers, white solid **91a** (60 mg, R_f = 0.72) in 44% yield, and **91b** (63 mg, R_f = 0.44) in 46% yield.

91a, ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 27.16 ppm. ^1H NMR (500 MHz, $CDCl_3$): δ 8.91 (br, s, H, NH), 7.65-7.23 (m, 5H, aromatic H), 7.40 (s, 1H, H-6), 6.70 (m, 1H, H-3-indole), 6.43 (dd, J = 9.2 Hz, 5.0 Hz, 1H, H-1'), 4.75 (m, 1H, H-3'), 4.50 (s, br., 1H, H-4'), 3.88 (m, 2H, HH'-5'), 2.25 (dd, J = 14 Hz, 5.5 Hz, H-2'), 1.90 (d, J = 17.5 Hz, 3H, CH_3P), 1.86 (s, 3H, CH_3C-5), 1.83 (m, 1H, H'-2'), 0.78 (s, 9H, $C(CH_3)_3$), 0.00 (ss, 6H, CH_3SiCH_3). MS (CI, NH_3): 534 ($M+H^+$, 1.8%), 476 (34.1%), 281 (98.6%).

91b, ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 26.57 ppm. ^1H NMR (500 MHz, $CDCl_3$): δ 8.31 (br, s, H, NH), 7.66-7.26 (m, 5H, aromatic H), 7.35 (s, 1H, H-6), 6.70 (m, 1H, H-3-indole), 6.46 (dd, J = 9.5 Hz, 5.5 Hz, 1H, H-1'), 4.75 (m, 1H, H-3'), 3.92 (s, br., 1H, H-4'), 3.50, 2.93 (m, 2H, HH'-5'), 2.70 (dd, J = 14 Hz, 5.5 Hz, H-2'), 2.10 (m, 1H, H'-2'), 1.94 (d, J = 17.5 Hz, 3H, CH_3P), 1.86 (s, 3H, CH_3C-5), 0.74 (s, 9H, $C(CH_3)_3$), -0.14, -0.19 (ss, 6H, CH_3SiCH_3).

Indole derivative 92

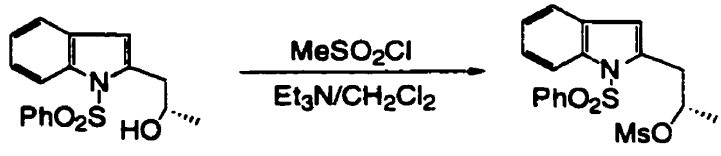


87 92

A solution of **87** (from Route A, 0.43 mmol) was treated with Beaucage's reagent (120 mg, 0.6 mmol) for ten minutes. Purification by flash chromatography (ethyl acetate) afforded light yellow solid indole derivative **92** (160 mg) in 69% yield. The two diastereomers of **92** could not be separated by silica gel column chromatography.

^{31}P NMR (109.3 MHz, $CDCl_3$): δ 82.94 ppm (42.8%), 82.83 ppm (57.2%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.0 (br, d, H, NH), 7.8-7.1 (m, 6H, aromatic H, H-6), 6.61 (m, 1H, H-3-indole), 6.41 (m, 1H, H-1'), 5.0 (m, 1H, H-3'), 4.5, 3.78 (m, 1H, H-4'), 3.9, 3.45, 3.1 (m, 2H, HH'-5'), 2.7, 2.08 (m, 2H, HH'-2'), 2.24 (2 × d, J = 17.5 Hz, 3H, CH_3P), 1.86 (2 × s, 3H, CH_3C-5), 0.8 (2 × s, 9H, $C(CH_3)_3$), 0.03, -0.10 (2 × d, 6H, CH_3SiCH_3).

(S)-(1-phenylsulfonylindol-2-yl)-isopropyl methanesulfonate 93



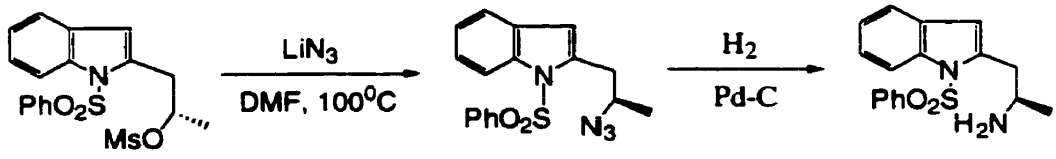
(S)-25 93

To a solution of (S)-(1-phenylsulfonylindol-2-yl)isopropanol **25** (3.7 g, 11.7 mmol) dichloromethane (30 ml) was added 1.0 ml of methanesulfonyl chloride (12.9 mmol) and 2.0 ml of triethylamine (14.4 mmol). The reaction mixture was stirred overnight, then washed with brine (2 × 40 ml), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The residue was purified by flash chromatography

(hexanes/ethyl acetate 2:1) to give a light red oil (S)-(1-phenylsulfonylindol-2-yl)-isopropanyl methanesulfonate **93** (4.6 g) in quantitative yield. $[\alpha]^{D}_{295}$ -38.98^{0} (c 0.935, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): 8.1-7.2 (m, 9H, aromatic H), 6.56 (s, 1H, H-3-indole), 5.19 (m, 1H, CHO), 3.34 (d, J = 6.2 Hz, 2H, CCH_2), 2.66 (s, 3H, SCH_3), 1.53 (d, J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 138.31, 137.31, 135.97, 134.07, 129.50, 129.41, 126.24, 124.97, 124.25, 120.75, 115.15, 113.28, 79.36, 37.64, 36.58, 21.59. MS (EI): 393 ($M^{+\cdot}$, 10.3%), 297 (100.0%), 157 (66.7%).

(R)-(1-phenylsulfonylindol-2-yl)-isopropylamine 95



93 **94** **95**

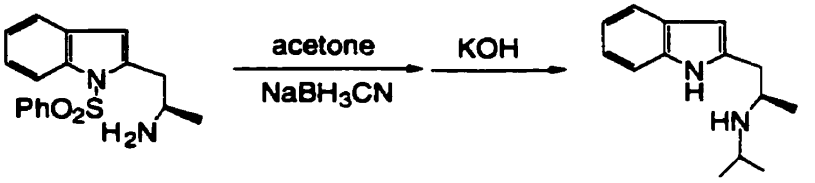
A solution of (S)-(1-phenylsulfonylindol-2-yl)-isopropanyl methanesulfonate **93** (4.7 g, 12 mmol) and LiN_3 (1.5 g, 30.6 mmol) in DMF (30 ml) was stirred 5 for hours at 100 ^{0}C. The solution was concentrated under vacuum, diluted with 50 ml of water and extracted with ethyl acetate (3 x 30 ml). The combined extracts was dried over anhydrous sodium sulfate and evaporated to give azide derivative **94** (4.37 g) as an amber oil.

The azide derivative **94** was dissolved in 30 ml of ethanol and shaken at RT under 30 PSI of hydrogen in the presence of Pd/C (200 mg, 10% Pd/C) for 3 hours. The catalyst was filtered off and washed with ethanol (5 ml). Evaporation of the solvent afforded light yellow sticky solid (R)-(1-phenylsulfonylindol-2-yl)-isopropylamine **95** (3.62 g) in 96% yield for the two steps. $[\alpha]^{D}_{295}$ 38.84^{0} (c 0.69, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): δ 8.1-7.1 (m, 9H, aromatic H), 6.45, 6.34 (s, 1H, H-3-indole), 3.99, 3.42 (m, 1H, CH_2CHN), 3.10, 2.91 (m, 2H, CCH_2), 1.92, 1.68 (2 x s, 4H, unknown), 1.53 (2 x d, J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 139.73, 139.57, 138.87, 138.80, 137.45, 137.33, 133.72, 133.69, 129.99, 129.82,

129.25, 129.22, 126.23, 124.25, 124.12, 123.86, 123.78, 120.35, 120.30, 115.13, 115.08, 111.88, 111.20, 55.06, 46.88, 40.04, 37.67, 30.98, 29.31, 23.61, 21.38, 18.29. MS (EI): 314 ($M^{+\cdot}$, 2.7%), 271 (100.0%), 130 (80.8%). MS (CI, NH_3): 315 ($M+H^+$, 23.4%), 271 (100.0%).

(R)-N-isopropyl-(indol-2-yl)isopropylamine 96



95 **96**

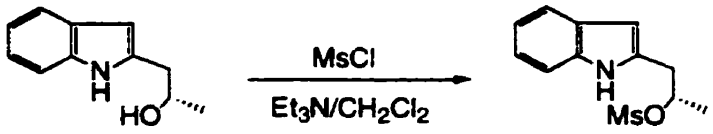
To a solution of (R)-(1-phenylsulfonylindol-2-yl)-isopropylamine **95** (3.62 g, 11.6 mmol) in methanol (30 ml) was added 10 ml of acetone followed by sodium cyanoborohydride (1.5 g, 23 mmol). The pH of the reaction mixture was adjusted to 6 by slow addition of acetic acid and the mixture was stirred overnight at RT. The mixture was then concentrated on a rotary-evaporator, and the resulting oil was redissolved in ethyl acetate (20 ml), washed with saturated sodium bicarbonate (2 x 20 ml) and brine (2 x 15 ml), dried over sodium sulfate and evaporated to give a sticky oil.

This oil was dissolved in 30 ml of methanol/water (3:1) and 3.0 g of KOH (54 mmol) was added. The mixture was refluxed for 5 hours. The solution was concentrated on a rotary evaporator, diluted with water (40 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were dried over anhydrous sodium sulfate, evaporated to give a sticky solid. This crude product was purified by flash chromatography (acetone) to give white solid (R)-N-isopropyl-(indol-2-yl)isopropylamine **96** (0.55 g) in 22% yield for the two steps, m.p. 68 - 69 ^{0}C. $[\alpha]^D_{295}$ -17.23^0 (c 0.545, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): δ 9.6 (br. s, 1H, NH), 7.5-7.0 (m, 4H, aromatic H), 6.21 (s, 1H, H-3-indole), 3.13 (m, 1H, CH_2CHO), 3.10, 2.99 (heptet, J = 6.2 Hz, 1H, NCH), 2.9, 2.7 (m, 2H, CCH_2), 1.11 (3 x d, J = 6.2 Hz, 9H, CH_3, $(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 138.30, 135.85, 128.52, 120.79, 119.71, 119.31, 110.65,

100.23, 50.27, 45.73, 35.07, 24.08, 22.96, 20.77. MS (CI, NH_3): 217 ($M+H^+$, 100.0%), 130 (49.0%).

(S)-indol-2-ylisopropyl methanesulfonate 98

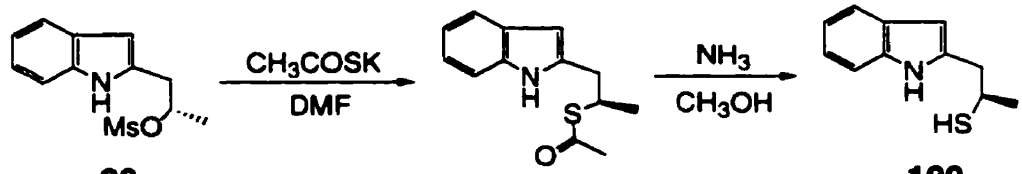


(S)-**26** **98**

To a solution of (S)-indol-2-ylisopropanol **26** (1.6 g, 9.1 mmol) in dichloromethane (30 ml) was added 1.0 ml of methanesulfonyl chloride (12.9 mmol) and 2.0 ml of triethylamine (14.4 mmol). The reaction mixture was stirred overnight, then washed with brine (2 × 40 ml), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The residue was purified by flash chromatography (hexanes/ethyl acetate 2:1) to give white solid (S)-indol-2-ylisopropyl methanesulfonate **93** (2.3 g) in quantitative yield.

^1H NMR (270 MHz, $CDCl_3$): δ 8.31 (br. s, 1H, NH), 7.5-7.0 (m, 4H, aromatic H), 6.33 (s, 1H, H-3-indole), 5.05 (m, 1H, CHO), 3.14 (m, 2H, CH_2), 2.74 (s, 3H, SCH_3), 1.45 (d, J = 6.1 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.24, 133.32, 128.39, 121.84, 120.16, 119.97, 110.82, 102.38, 79.55, 38.30, 35.64, 20.90.

(R)-indol-2-ylisopropanethiol 100

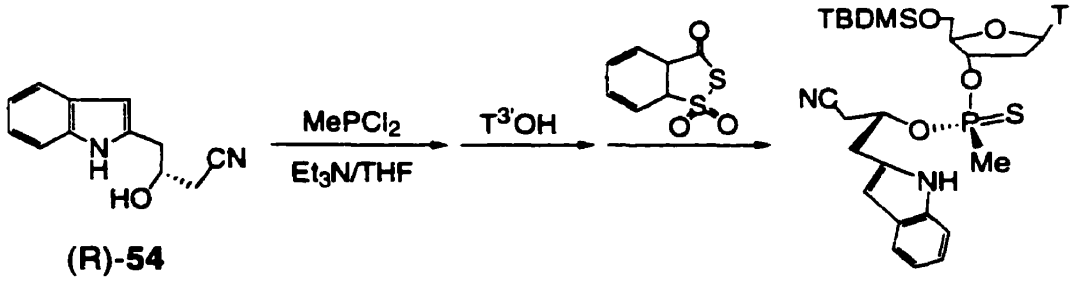


98 **99** **100**

A solution of (S)-indol-2-ylisopropyl methanesulfonate **98** (2.04 g, 8.06 mmol) in DMF (30 ml) containing 2.0 g of potassium thioacetate (17.5 mmol) was stirred for 5 hours at 110 ^{0}C. The solution was concentrated under vacuum, diluted with 50 ml of water and extracted with ethyl acetate (3 x 30 ml). The combined extracts was dried over anhydrous sodium sulfate and evaporated to give an amber oil. This crude thioester **99** was dissolved in 20 ml of methanol and ammonia gas was bubbled into the solution for 5 minutes at 0 ^{0}C. The solution was stirred for 2 hours at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (hexanes/ethyl acetate 1:2) to give (R)-indol-2-ylisopropanethiol **100** (0.87 g) in 57% yield as light yellow sticky solid. $[\alpha]^D_{295}$ 17.76^0 (c 1.1, ethyl acetate).

^{1}H NMR (270 MHz, $CDCl_3$): δ 7.78 (br. s, 1H, NH), 7.5-7.0 (m, 4H, aromatic H), 6.29 (s, 1H, H-3-indole), 3.15 (m, 2H, CHO, C**H**H'), 2.89 (m, 1H, CH**H**'), 1.30 (d, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.24, 135.98, 128.53, 121.54, 120.02, 119.95, 110.75, 101.65, 46.49, 35.01, 20.53. MS (CI, NH_3): 192 ($M+H^+$, 81.7%), 158 (44.3%), 130 (100.0%).

Rp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (R)-(1-cyano-3-indol-2'-yl)-isopropyl methylthiophosphonates 103



(R)-**54** Rp-**103**

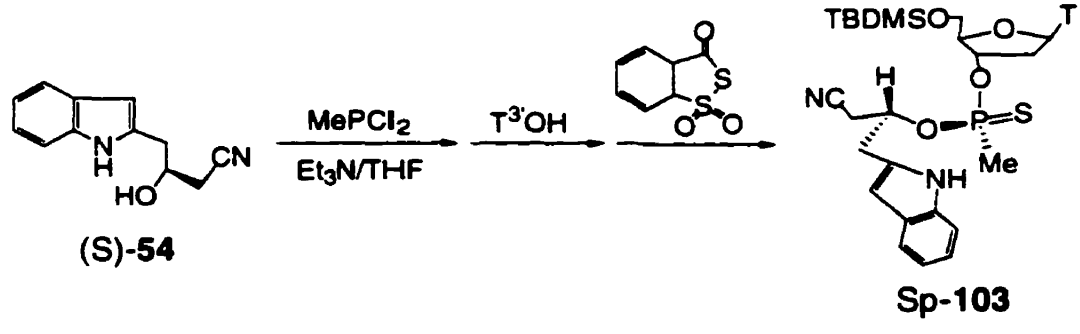
To a solution of (R)-3-hydroxy-4-(2-indolyl)butyronitrile **54** (200 mg, 1.0 mmol) in THF (10 ml) containing triethylamine (0.4 ml, 3 mmol) was added 0.10 ml of dichloromethylphosphorine (90%, 1.2 mmol) *via* a micro-syringe at -78 ^{0}C. The reaction mixture was stirred for 1 hour at room temperature. A solution of 5-O'-TBDMS-thymidine

(356 mg, 1.0 mmol) in CH_2Cl_2 (0.5 ml) was introduced *via* a syringe followed by 0.30 ml of DBU (2.0 mmol). The reaction mixture was stirred for 30 minutes, then a solution of Beaucage's reagent (300 mg, 1.5 mmol) in THF (0.5 ml) was introduced. After 5 minutes, the solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate) to give white solid methylthiophosphonate **103** (134 mg) in 22% yield, m.p. 71 - 72 ^{0}C

Two diastereoisomers of **103** were obtained in a ratio of 6 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 96.87 (86%, Rp-**103**), 98.53 (14%). The following NMR spectra were assigned for the major one.

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.01 (br s, 1H, NH-indole), 8.97 (br s, 1H, NH-T), 7.5- 7.0 (m, 4H, aromatic H), 7.35 (s, 1H, H-6), 6.37 (s, 1H, H-3-indole), 6.18 (dd, 1H, J = 9.0, 5.5 Hz, H-1'), 5.00 (m, 2H, H-3', CHOP), 4.09 (m, 1H, H-4'), 3.80 (m, 2H, HH'-5'), 3.26 (m, 2H, CH_2C), 2.88, 2.69 (m, 2H, CH_2CN), 2.04, 1.65 (m, 2H, HH'-2'), 1.93 (d, J = 15.0 Hz, 3H, PCH_3), 1.92 (s, 3H, CH_3C-5), 0.91 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 164.01 (C-4), 150.68 (C-2), 136.07, 134.77, 132.03, 128.35, 121.60, 119.89, 119.70, 116.01, 111.14, 110.85, 101.87, 85.89 (d), 84.69, 77.58 (d), 71.98 (d), 62.96, 39.16 (d), 33.73 (d), 35.74, 23.67 (d), 23.45, 22.52, 18.14, 12.37, -5.53, -5.58. MS (FAB, NBA): 633 ($M+H^+$, 68.2%), 655 ($M+Na^+$, 16.5%).

Sp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl (S)-(1-cyano-3-indol-2-yl)-isopropyl methylthiophosphonates 103



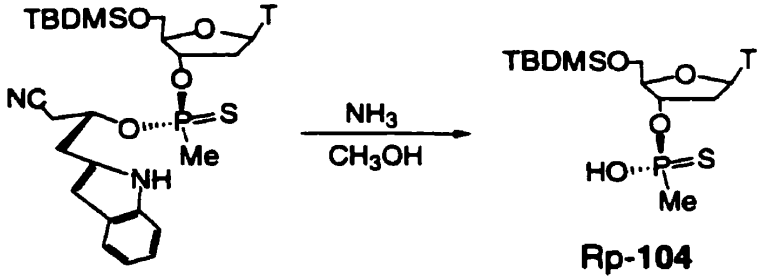
(S)-**54** Sp-**103**

Using the same procedure as described for the synthesis of Rp-**103**, (S)-3-hydroxy-4-(2-indolyl)butyronitrile **54** (200 mg, 0.2 mmol) provided white solid methylthiophosphonate **103** (272 mg) in 43% yield.

Two diastereoisomers of **103** were obtained in a ratio of 6 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 97.82 (86.3%, Sp-**103**), 98.30 (13.7%). The following NMR spectra were assigned for the major one.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.88 (br s, 1H, NH-indole), 8.41 (br s, 1H, NH-T), 7.5- 7.0 (m, 4H, aromatic H), 7.41 (s, 1H, H-6), 6.37 (s, 1H, H-3-indole), 6.29 (dd, 1H, J = 9.5, 5.5 Hz, H-1'), 5.07 (m, 1H, H-3'), 5.03 (m, 1H, CHOP), 3.87 (m, 1H, H-4'), 3.54 (m, 2H, HH'-5'), 3.29, 3.18 (m, 2H, CH_2C), 2.84, 2.69 (m, 2H, CH_2CN), 2.04, 2.05 (m, 2H, HH'-2'), 1.96 (d, J = 15.5, 3H, PCH_3), 1.90 (s, 3H, CH_3C-5), 0.89 (s, 9H, $SiC(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.45 (C-4), 150.17 (C-2), 136.08, 134.82, 131.62, 128.13, 122.02, 120.06, 120.02, 115.87, 111.10, 110.71, 102.42, 85.73 (d), 84.54, 77.15 (d), 71.97 (d), 62.69, 39.22 (d), 33.73 (d), 25.74, 23.66, 23.62 (d), 22.68, 18.12, 12.34, -5.56, -5.62.

Rp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl methylthiophosphonates 104



Rp-103 Rp-104

130 mg of methylthiophosphonate diester **103** (the major Rp-**103**, with the other isomer in a ratio of 6:1) was dissolved in 20 ml methanol and ammonia gas was bubbled into the solution for 5 minutes at 0 °C. The solution was stirred for half an hour at room temperature. The solvent was evaporated and the residue was purified by flash

chromatography (methanol/acetone 1:5) to give white solid methylthiophosphonate monoester **104** (73.5 mg) in 80% yield, m.p. 186 - 187 ^{0}C.

Two diastereoisomers of **104** were obtained in a ratio of 5 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 77.93 (83%, Rp-**104**), 78.13 (17%, Sp-**104**). The following NMR spectra were assigned for the major one.

^{1}H NMR (500 MHz, CD_3OD): δ 7.58 (s, 1H, H-6), 6.19 (m, 1H, H-1'), 5.11 (m, 1H, H-3'), 4.11 (m, 1H, H-4'), 3.86 (m, 2H, HH'-5'), 2.47, 2.07 (m, 2H, HH'-2'), 1.80 (s, 3H, CH_3C-5), 1.56 (d, J = 15.0 Hz, 3H, PCH_3), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 166.18 (C-4), 152.20 (C-2), 137.20, 111.46, 88.10 (d), 86.36, 75.90(d), 64.58, 40.86, 26.43, 25.33, 24.46, 19.17, 12.59, -5.21, -5.28. MS (FAB, NBA): 473 ($M+Na^+$, 48.6%).

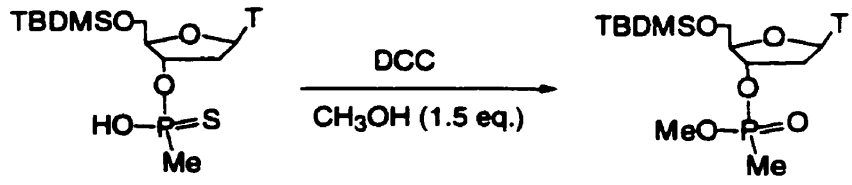
Sp-(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl methylthiophosphonates 104

Using the same procedure as described for the synthesis of Rp-**104**, the deprotection of Sp-**103** (272 mg) with ammonia afforded colorless sticky solid Sp-**104** (166.2 mg) in 86% yield.

Two diastereoisomers of **104** were obtained in a ratio of 5.3 : 1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 76.70 (84%, Sp-**104**), 76.28 (16%, Rp-**104**). The following NMR spectra were assigned for the major one.

^{1}H NMR (500 MHz, CD_3OD): δ 7.60 (s, 1H, H-6), 6.21 (dd, J = 9.0 Hz, 5.5 Hz, 1H, H-1'), 5.07 (dd, J = 11.0 Hz, 6.0 Hz, 1H, H-3'), 4.26 (m, 1H, H-4'), 3.93, 3.85 (m, 2H, HH'-5'), 2.34, 2.08 (m, 2H, HH'-2'), 1.80 (s, 3H, CH_3C-5), 1.58 (d, J = 15.0 Hz, 3H, PCH_3), 0.87 (s, 9H, $SiC(CH_3)_3$), 0.09 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 166.28 (C-4), 152.24 (C-2), 137.23, 111.36, 88.49 (d), 86.47, 76.84(d), 64.68, 40.66 (d), 26.42, 25.31, 24.45, 19.18, 12.58, -5.20, -5.26.

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl methyl methylphosphonate 105



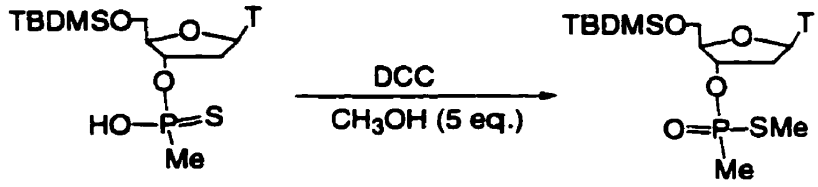
104 **105**

A solution of methylphosphorothionate monoester **104** (Rp:Sp. 1:1) (30 mg, 0.067 mmol)) in THF (1.5 ml) containing 4.0 µl of methanol (0.10 mmol) and DCC (55 mg, 0.26 mmol) was warmed at 50 ^{0}C over night. The solvent was evaporated and the residue was purified by chromatography (ethyl acetate) to give methylphosphonate **105** (18 mg) in 60% yield.

Two diastereomers of **105** were obtained as established by ^{31}P NMR in a ratio of 1:1, which could not be separated by silica gel column chromatography. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 32.87 ppm, 32.76 ppm.

^{1}H NMR (500 MHz, CD_3OD): δ 7.43 (2 × s, 1H, H-6), 6.38 (m, 1H, H-1'), 5.00 (m, 1H, H-3'), 4.22 (m, 1H, H-4'), 3.85 (m, 2H, HH'-5'), 3.72 (2 × d, 2.48, 2.12 (m, 2H, HH'-2'), 2.33 (d, J = 13.5 Hz, 3H, $PSCH_3$), 1.92 (s, 3H, CH_3C-5), 1.85 (d, J = 15.5 Hz, 3H, PCH_3), 0.92 (s, 9H, $C(CH_3)_3$), 0.06 (s, 6H, $Si(CH_3)_2$). MS (FAB, NBA): 449 ($M+H^+$, 4.6%).

(5'-O-*tert*-butyldimethylsilyl)thymid-3'-yl methyl methylphosphorothionate 106



104 **106**

A solution of methylphosphorothioate monoester **104** (Rp:Sp, 2:1) (30 mg, 0.067 mmol) in THF (1,5 ml) containing 14.0 µl of methanol (0.35 mmol) and DCC (55 mg, 0.26 mmol) was warmed at 50 ^{0}C over night. The solvent was evaporated and the residue was purified by chromatography (ethyl acetate) to give two diastereomers of **106a** and **106b** in 19% (6 mg) and 32% (10 mg) yield respectively.

106a, ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 57.17 ppm. ^{1}H NMR (500 MHz, CD_3OD): δ 8.11 (br. s, 1H, NH), 7.52 (s, 1H, H-6), 6.36 (dd, J = 9.0 Hz, 5.5 Hz, 1H, H-1'), 5.12 (m, 1H, H-3'), 4.36 (m, 1H, H-4'), 3.92 (m, 2H, HH'-5'), 2.48, 2.12 (m, 2H, HH'-2'), 2.33 (d, J = 13.5 Hz, 3H, $PSCH_3$), 1.92 (s, 3H, CH_3C-5), 1.85 (d, J = 15.5 Hz, 3H, PCH_3), 0.92 (s, 9H, $C(CH_3)_3$), 0.06 (s, 6H, $Si(CH_3)_2$).

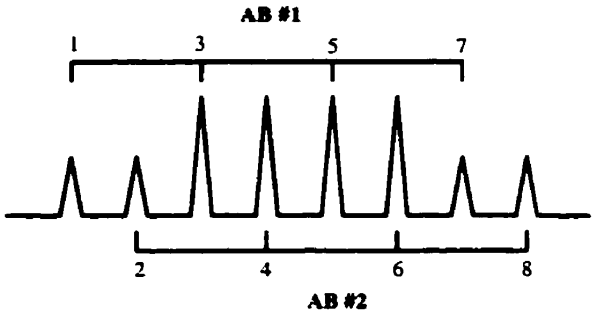
106b, ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 56.36 ppm. ^{1}H NMR (500 MHz, CD_3OD): δ 8.27 (br. s, 1H, NH), 7.43 (s, 1H, H-6), 6.41 (dd, J = 9.0 Hz, 5.5 Hz, 1H, H-1'), 5.18 (m, 1H, H-3'), 4.19 (m, 1H, H-4'), 3.86 (m, 2H, HH'-5'), 2.48, 2.16 (m, 2H, HH'-2'), 2.34 (d, J = 13.5 Hz, 3H, $PSCH_3$), 1.90 (s, 3H, CH_3C-5), 1.83 (d, J = 15.5 Hz, 3H, PCH_3), 0.92 (s, 9H, $C(CH_3)_3$), 0.06 (s, 6H, $Si(CH_3)_2$). MS (FAB, NBA): 487 ($M+Na^+$, 7.1%), 465 ($M+H^+$, 5.2%).

Appendixes

Appendix I: Analysis of ABX Systems in ^1H NMR Spectra.

The chemical shifts and coupling constants of second order AB portions of ABX systems were calculated by the method shown below.[183]

The ABX spectrum is divided into two AB systems.



$J_{A,B} = (8 - 6) = (7 - 5) = (4 - 2) = (3 - 1)$

AB #1

$\nu_1 = (1 + 3 + 5 + 7)/4$

$(\Delta\nu_1)/2 = [(1 - 7)(3 - 5)]^{1/2}/2$

$\Delta 1^+ = \nu_1 + (\Delta\nu_1)/2$

$\Delta 1^- = \nu_1 - (\Delta\nu_1)/2$

$\nu_A = (\Delta 1^+ + \Delta 2^+)/2$

$J_{AX} = \Delta 1^+ - \Delta 2^+$

or

$\nu_A = (\Delta 1^+ + \Delta 2^-)/2$

$J_{AX} = \Delta 1^+ - \Delta 2^-$

AB #2

$\nu_2 = (2 + 4 + 6 + 8)/4$

$(\Delta\nu_2)/2 = [(2 - 8)(4 - 6)]^{1/2}/2$

$\Delta 2^+ = \nu_2 + (\Delta\nu_2)/2$

$\Delta 2^- = \nu_2 - (\Delta\nu_2)/2$

$\nu_B = (\Delta 1^- + \Delta 2^-)/2$

$J_{BX} = \Delta 1^- - \Delta 2^-$

or

$\nu_B = (\Delta 1^- + \Delta 2^+)/2$

$J_{BX} = \Delta 1^- - \Delta 2^+$

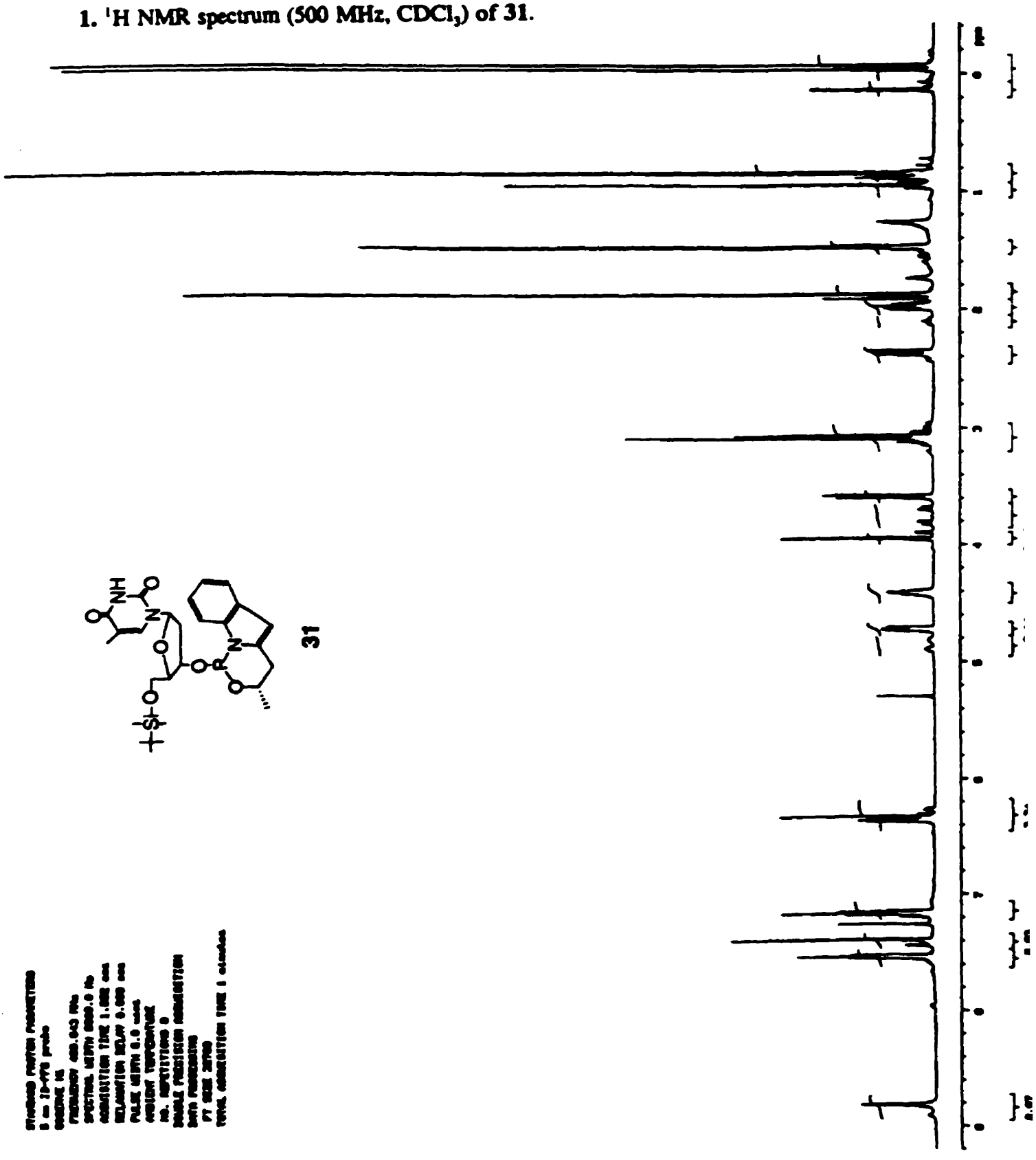
Two possible set of values are generated, but one gives unrealistic coupling constants.

---

[183] Becker, E. D. ed., *High Resolution NMR-Theory and Chemical Applications* **1980**, *Chapter 7*, Academic Press, Inc., London.

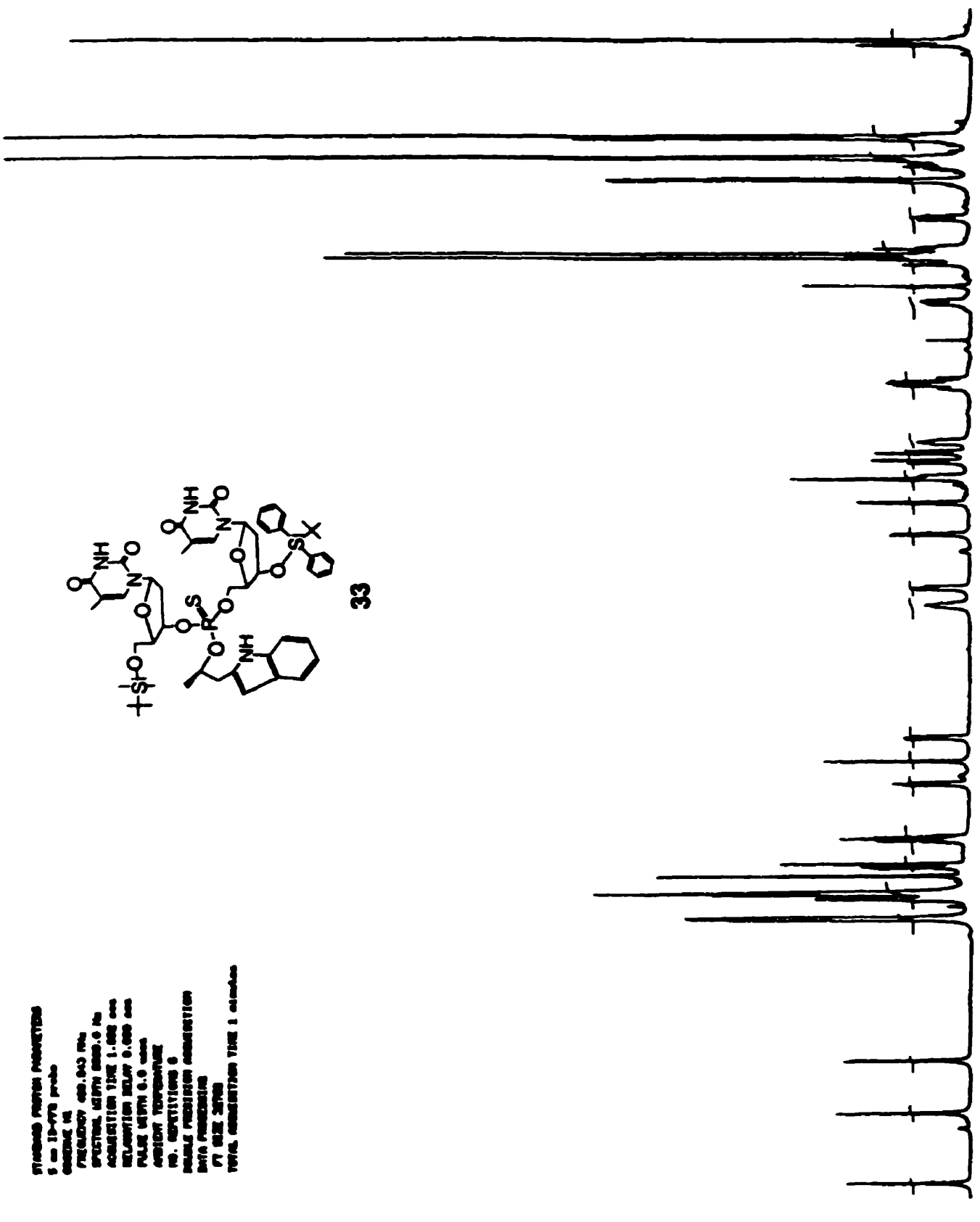
Appendix II: NMR Spectra

1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **31**.

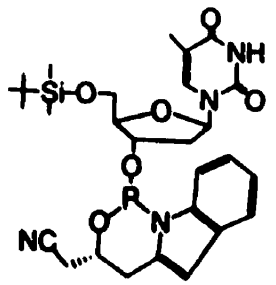


31

2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 33

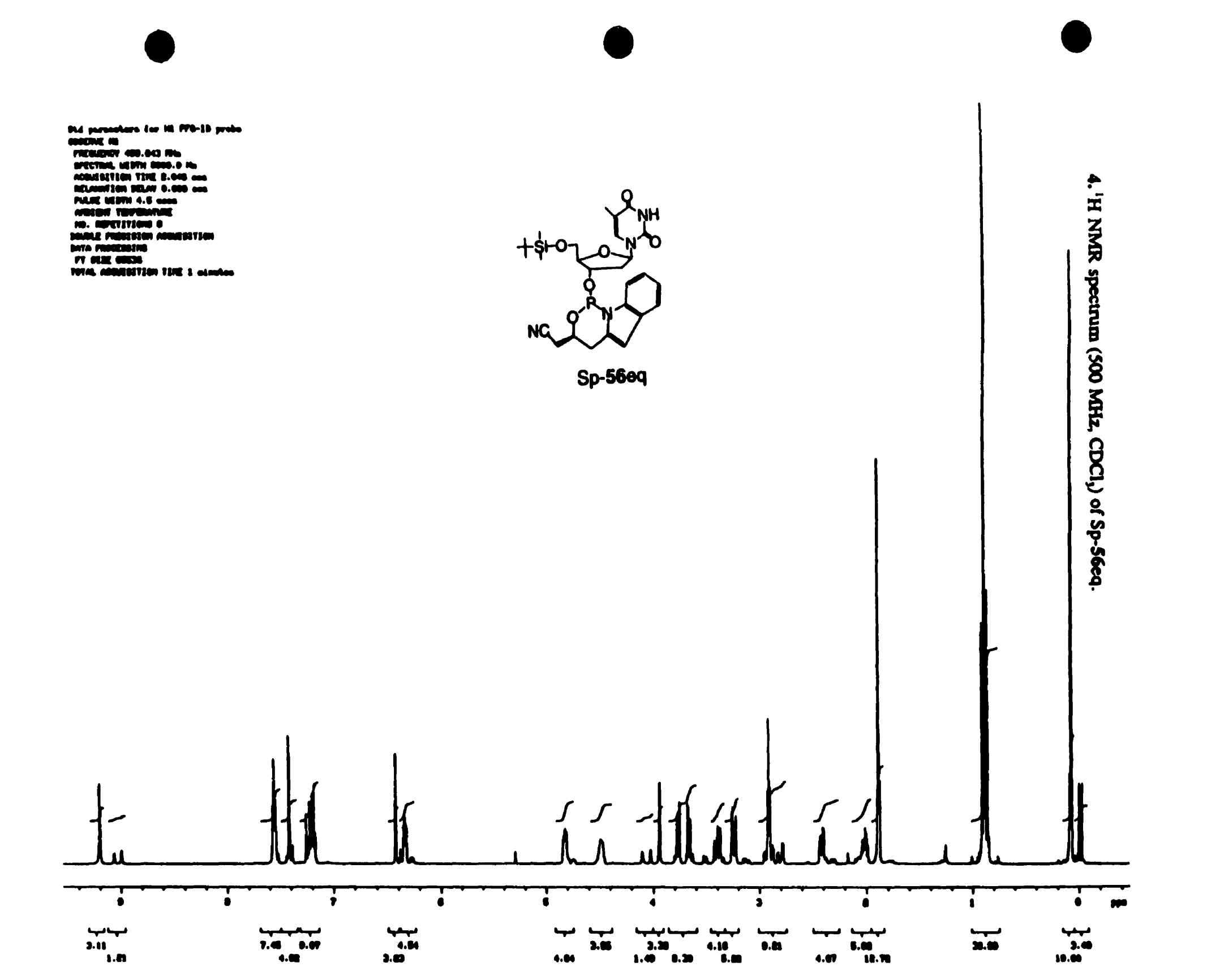


Std parameters for H1 PFG-ID probe
OBSERVE H1
FREQUENCY 400.043 MHz
SPECTRAL WIDTH 6000.0 Hz
ACQUISITION TIME 2.048 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 4.5 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 8
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes

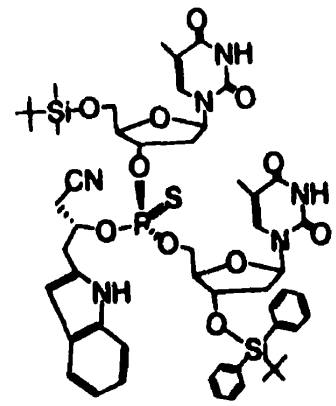


Rp-56eq

3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of Rp-56eq.


Sp-56eq
4. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of Sp-56eq.

Std parameters for HI PFG-ID probe
OBSERVE H1
FREQUENCY 499.843 MHz
SPECTRAL WIDTH 6000.0 Hz
ACQUISITION TIME 0.648 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 4.5 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 8
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes



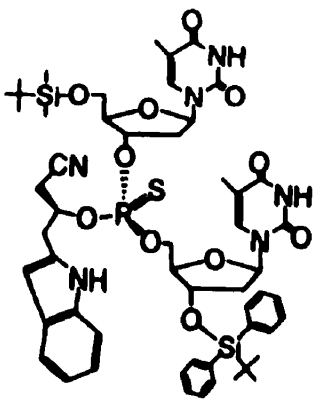
Rp-58

5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of Rp-58.

Std parameters for Nl PFG-ID probe

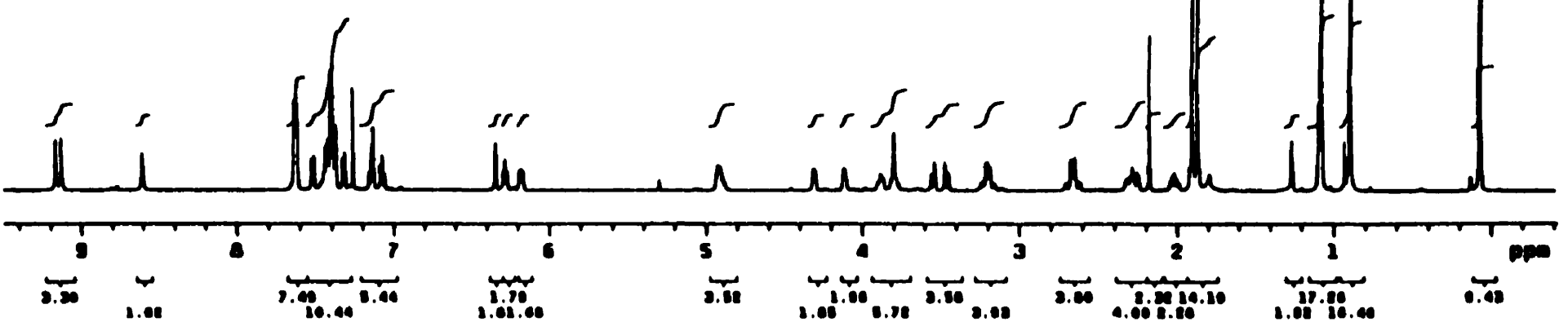
Solvent: CDCl3
Ambient temperature
File: N_1nCNPTTA
UNITY-500 "data500"

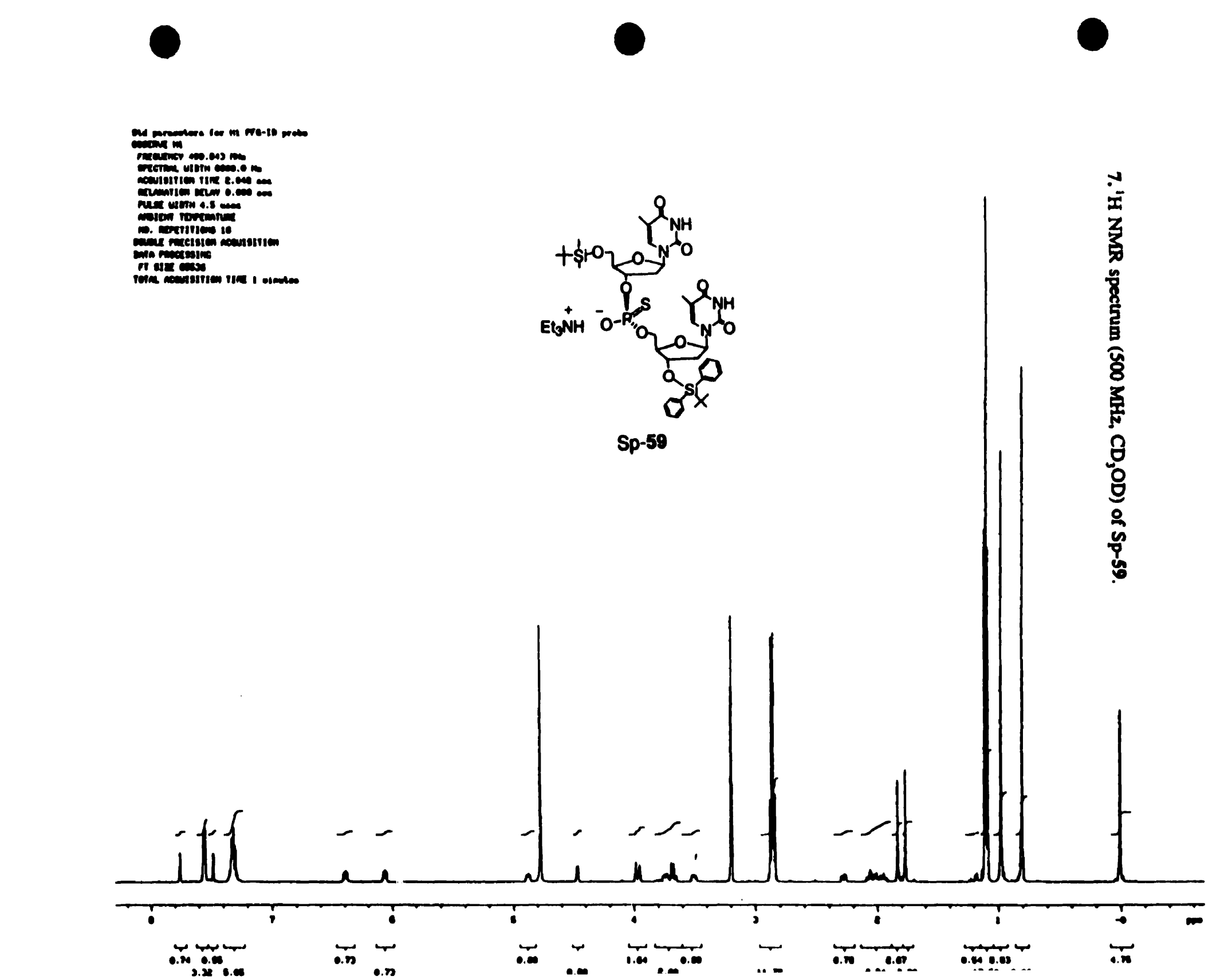
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
8 repetitions
OBSERVE Nl, 499.8408813 MHz
DATA PROCESSING
FT size 65536
Total time 1 minute



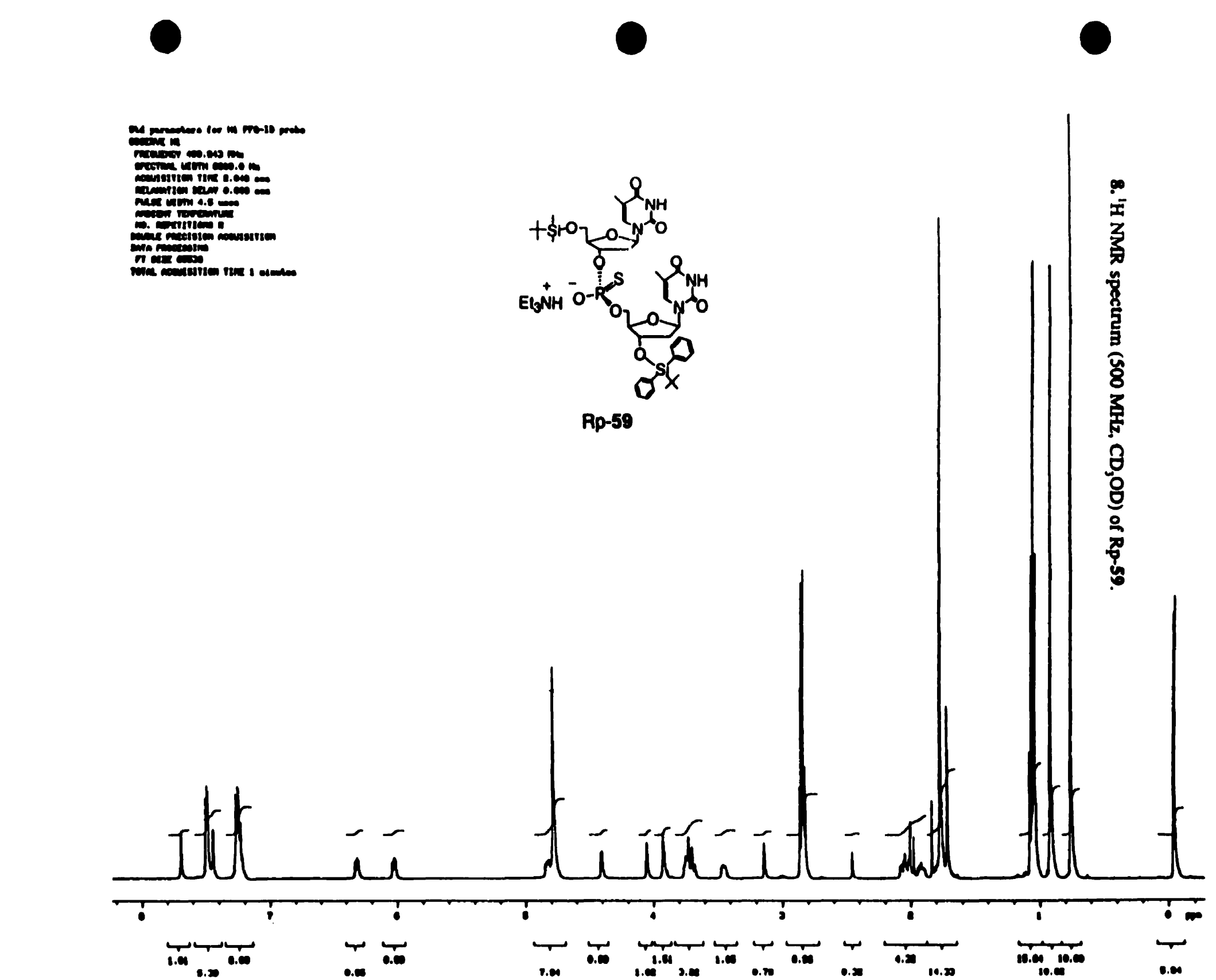
Sp-58

6. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of Sp-58.

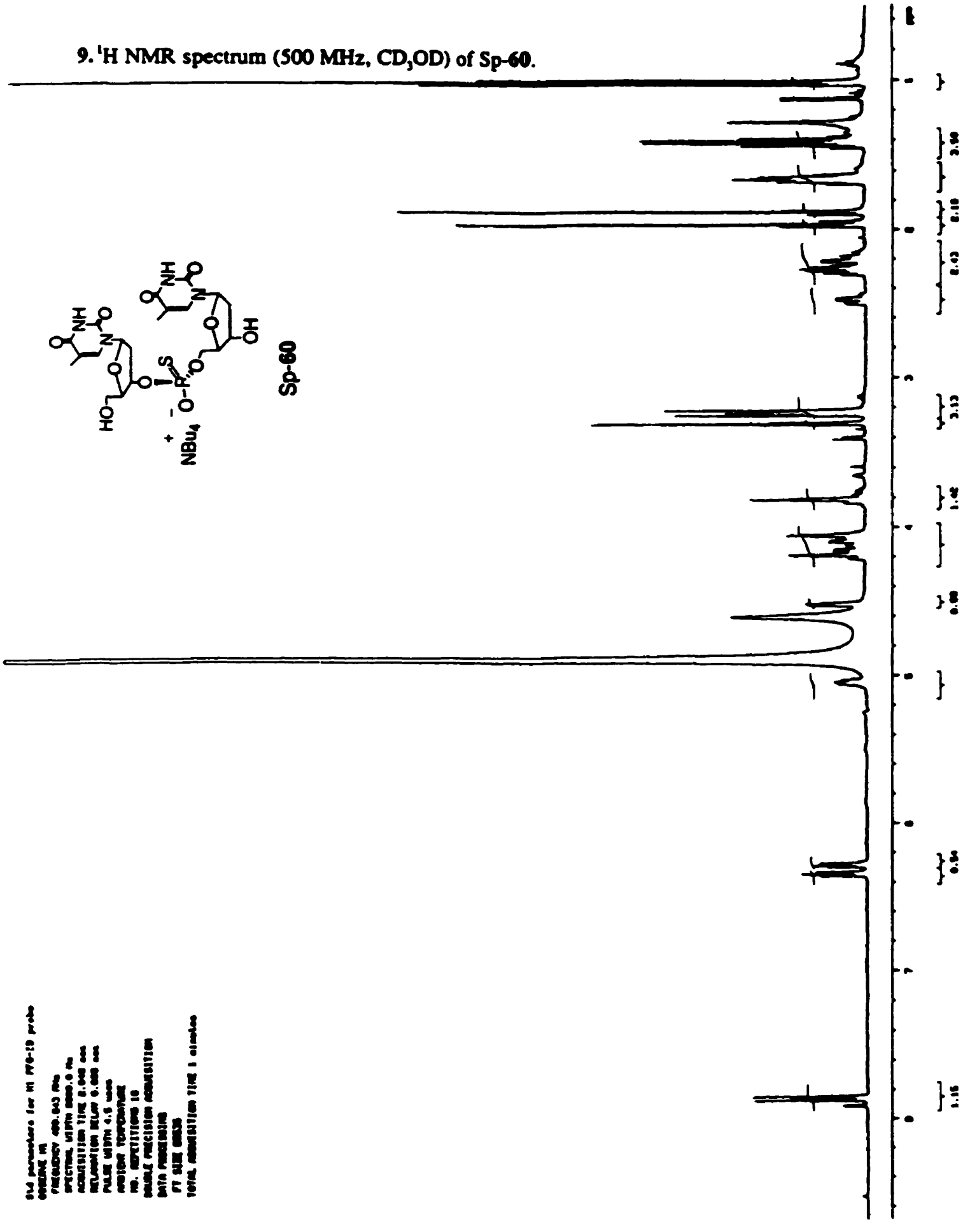


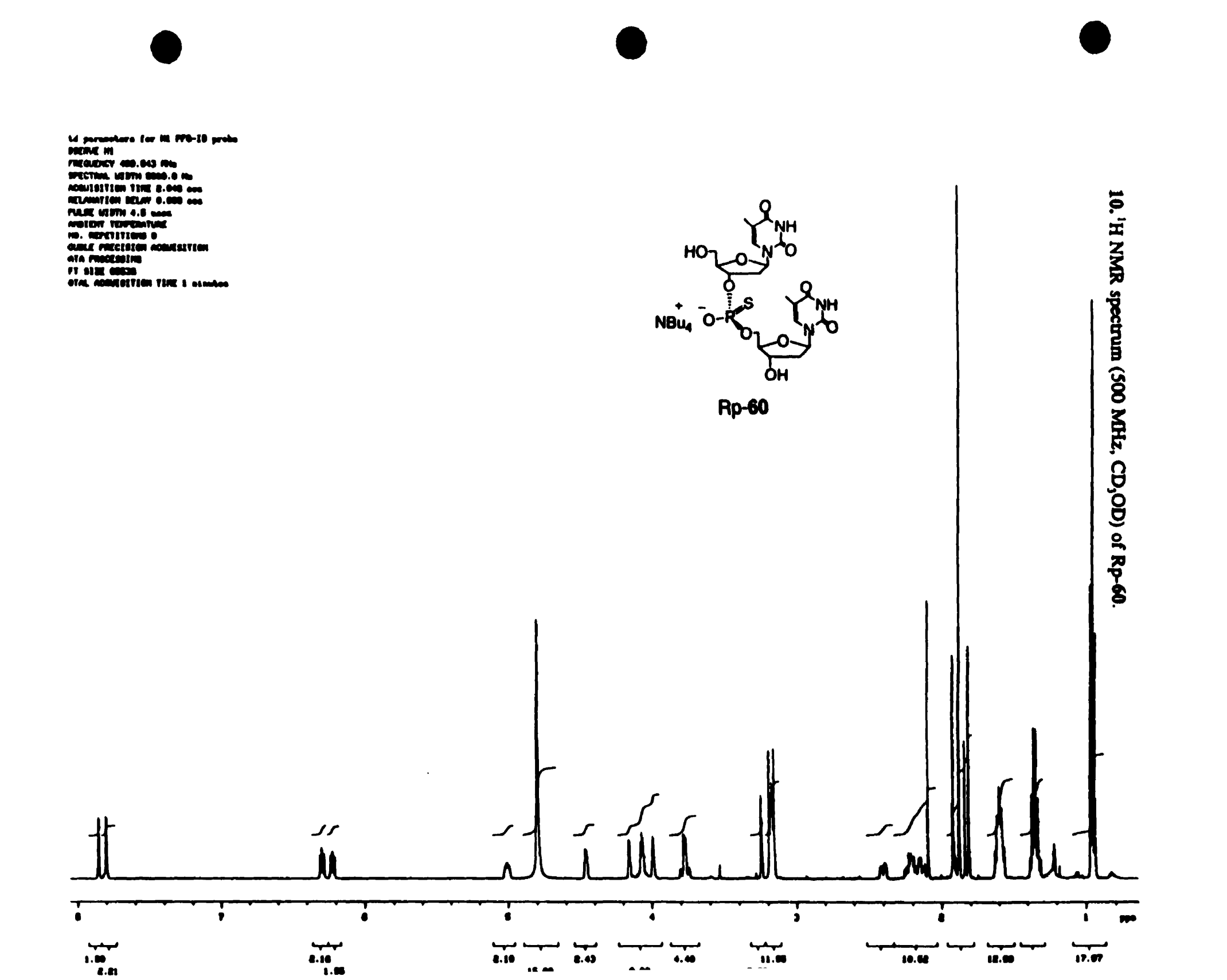


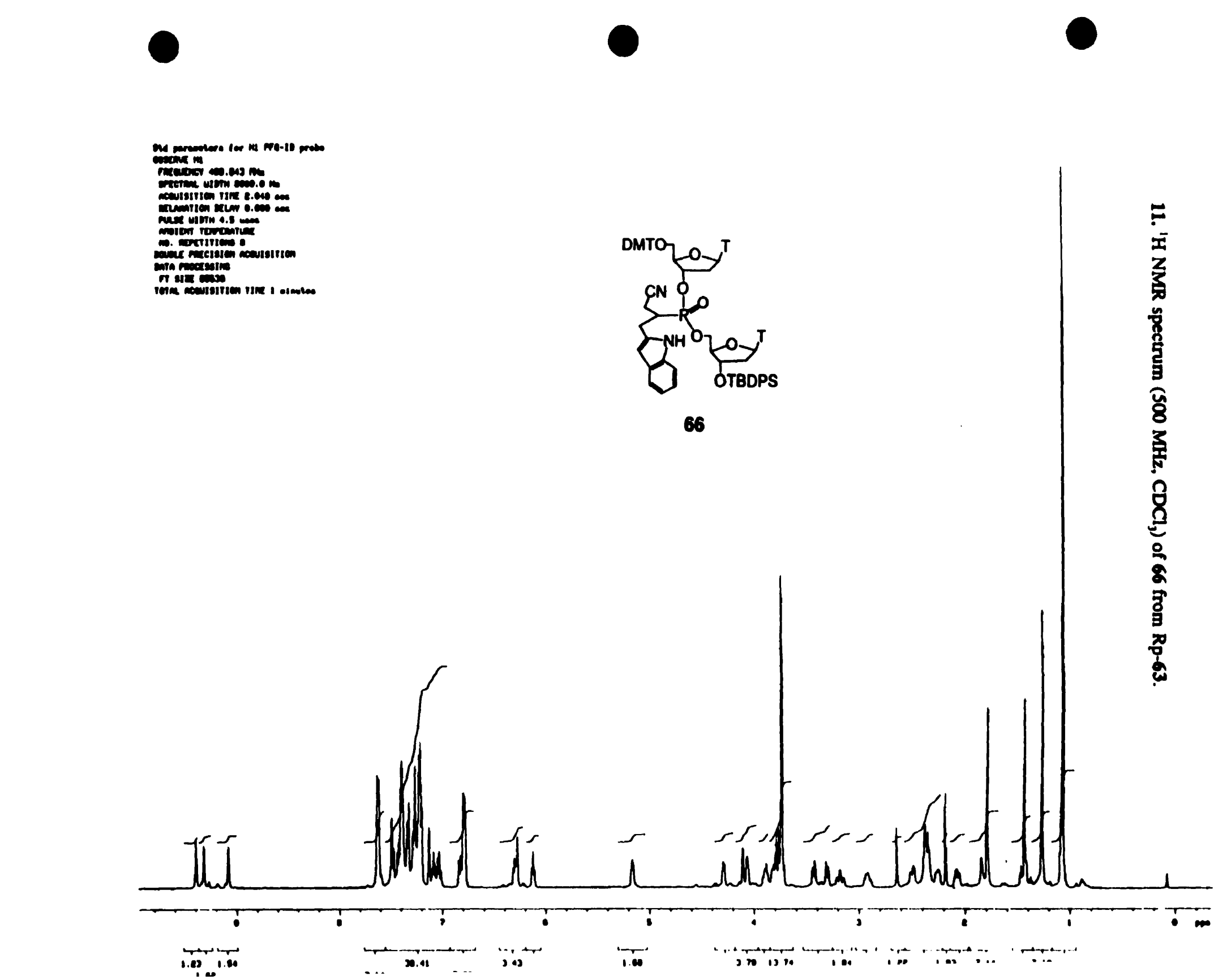
7. ^{1}H NMR spectrum (500 MHz, CD_3OD) of **Sp-59**.


Et3NH
NH
NH
Rp-59
8. ¹H NMR spectrum (500 MHz, CD3OD) of Rp-59.

9. ^{1}H NMR spectrum (500 MHz, CD_3OD) of Sp-60.

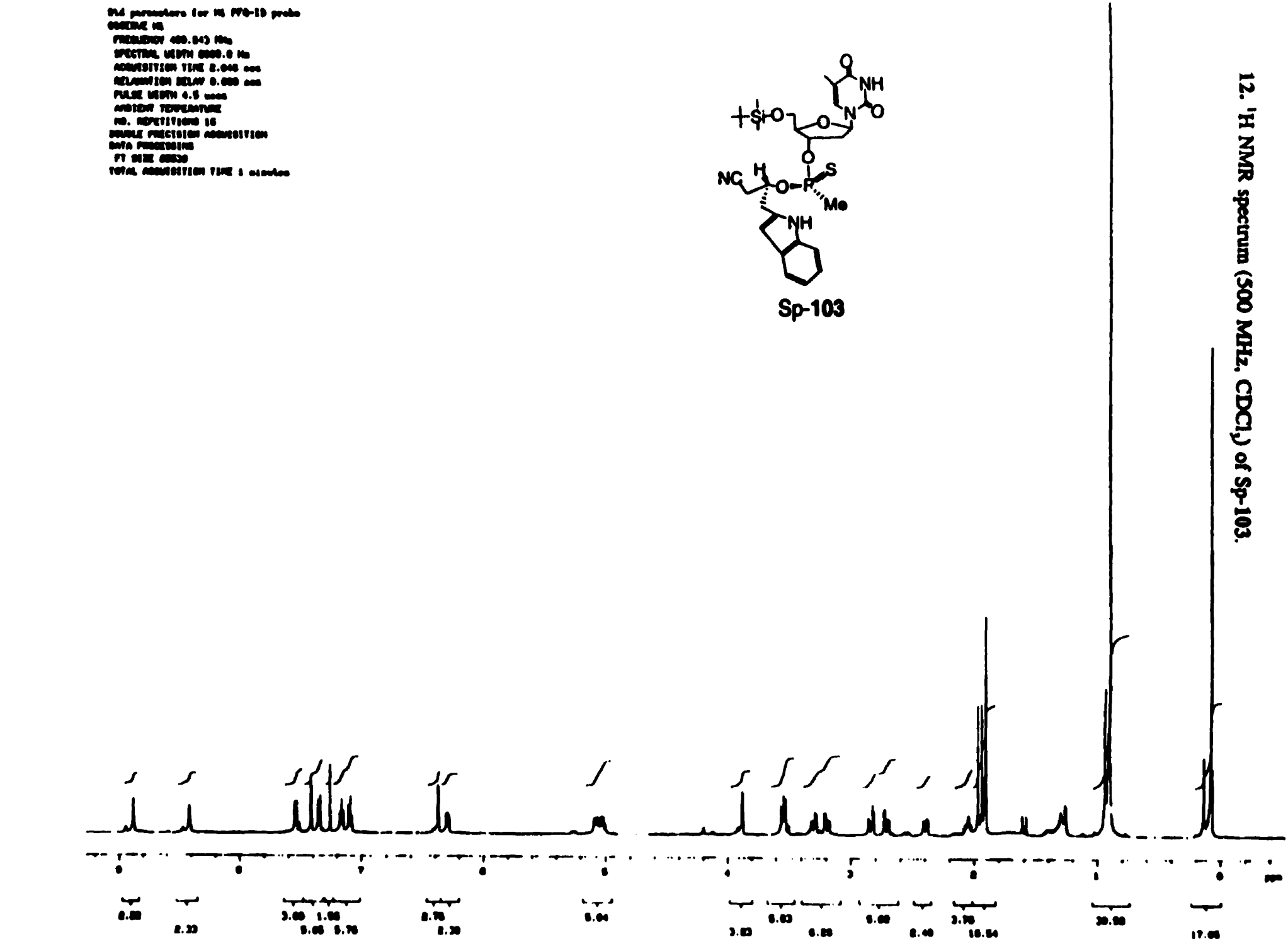



HO
NH
O
N
O
NBu4
O-P
S
Rp-60
10. ¹H NMR spectrum (500 MHz, CD3OD) of Rp-60.



66

11. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **66** from Rp-**63**.



12. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of Sp-103.

13. ^{1}H NMR spectrum (500 MHz, CD_3OD) of Sp-104.