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Diastereoselective synthesis of phosphite triesters and phosphorothioates

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements of the degree of Doctor of Philosophy

Department of Chemistry

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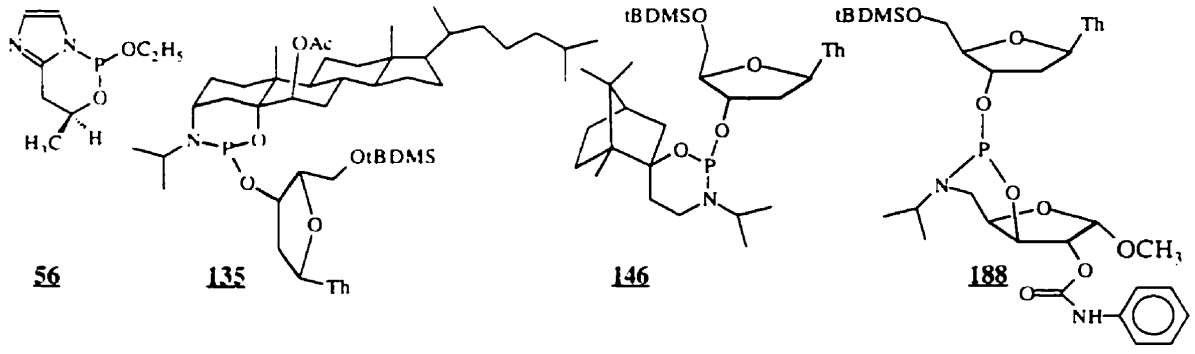
Abstract

The diastereoselective synthesis of phosphite triesters and related phosphorothioate triesters and diesters has been investigated, with the goal of synthesizing diastereomerically pure DNA phosphorothioates.

Towards this end, the elaboration of a new heterobicyclic structure, imidazo-oxazaphosphorine such as **56**, is reported. This unstable intermediate led to the highly diastereoselective synthesis of simple phosphite triesters upon reaction with various alcohols.

Two new types of sterically hindered chiral oxazaphosphorinanes **135** and **146** were then synthesized from cholesterol and camphor respectively. These structures, derived from γ-aminoalcohols possessing a tertiary alcohol function, could be isolated and characterized. They revealed very reactive in acidic conditions and led to rearrangements.

Finally, oxazaphosphorinane **188** derived from 1,2-O-isopropylidene-D-xylofuranose, was synthesized and characterized. The introduction of a participating group adjacent to the leaving phosphorothioate group led to the fast release of the phosphorothioate moiety. This new chiral auxiliary was successfully used as a precursor in the diastereoselective synthesis of a T-T phosphorothioate dimer, in a diastereomeric ratio of 28.5:1.



56 **135** **146** **188**

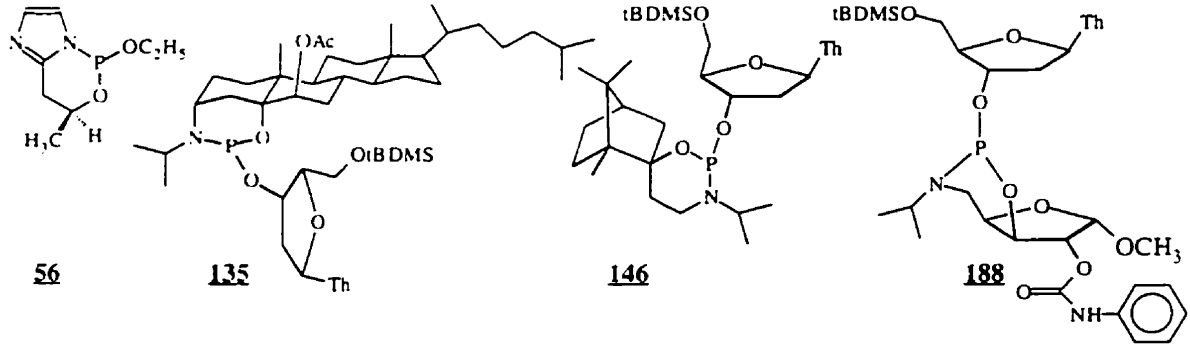
Résumé

Nous avons étudié la synthèse diastéréosélective de triesters de phosphite, ainsi que les triesters et diesters de phosphorothioate dérivés, avec pour objectif d'élaborer une nouvelle voie de synthèse diastéréosélective de phosphorothioates d'ADN.

Une structure hétérobicyclique nouvelle a été synthétisée, l'imidazo-oxazaphosphorine telle que **56**. Cet intermédiaire, dont la conformation a été étudiée, nous a permis de synthétiser, de façon hautement diastéréosélective, plusieurs triesters de phosphite simples ainsi que les triesters de phosphorothioate correspondants par réaction avec des alcools.

Puis, deux nouvelles oxazaphosphorinanes encombrées **135** et **136** ont été synthétisées à partir du cholestérol et du camphre respectivement. Ces structures, formées à partir d'alcools tertiaires γ-aminés, ont pu être isolées et caractérisées. Elles se révélèrent très réactives en milieu acide et conduisirent à des réarrangements.

Finalement, l'oxazaphosphorinane **188** a été synthétisée à partir du 1,2-O-isopropylidène-D-xylofuranose et caractérisée. L'introduction d'un groupe participant adjacent au groupe phosphorothioate partant a permis l'élimination rapide du groupement phosphorothioate. Ce nouvel auxiliaire chiral a mené à la synthèse diastéréosélective d'un dimère phosphorothioate T-T, dans un rapport diastéréomérique de 28.5 à 1.



56 **135** **146** **188**

Acknowledgments

First, I would like to express my gratitude to Prof. George Just for his advice and guidance throughout this work, as well as for providing many ideas necessary to carry out this work.

I would also like to thank Dr. M. J. Damha for helpful discussions on several occasions.

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Last but not least, my thanks go to my wife Myriam for her support in various ways and many occasions, as well as my family for encouragement throughout these studies.

LIST OF ABBREVIATIONS

Ac: acetyl

Ad: adenine

AIDS: acquired immuno-deficiency syndrome

APT: attached proton test (NMR sequence)

Ar: aryl

asDNA: *antisense* deoxyribonucleic acid

atm: atmosphere

b: broad (NMR)

B: base

BDT: 1,3-benzodithiol-2-yl

Bn: benzyl

b.p.: boiling point

Bu: *n*-butyl

Bz: benzoyl

c: concentration (for the measurement of optical rotation)

C: Celsius

calc.: calculated

cat.: catalytic amount

Cbz: carboxybenzyloxy

CI: chemical ionization

COSY: correlation spectroscopy

CPG: controlled pore glass

Cy: cytosine

δ: chemical shift (NMR)

dA: 2'-deoxyadenosine

DBU: 1,8-diazabicyclo[5,4,0]undec-1-ene

dC: 2'-deoxycytidine

d.e.: diastereomeric excess

dG: 2'-deoxyguanosine

DMAP: N,N-dimethyl-4-aminopyridine

DMF: dimethylformamide

DMSO: dimethylsulfoxide

DMTr: dimethoxytrityl (4,4'-dimethoxytriphenylmethyl)

DNA: deoxyribonucleic acid

dT: 2'-deoxythymidine

E. coli: *Escherichia Coli*

EI: electronic ionization

eq.: equivalent(s)

Et: ethyl

EtOAc: ethyl acetate

FAB: fast atom bombardment

g: gram(s)

Gu: guanine

h: heptet (NMR)

h.: hour(s)

HETCOR (hetero correlation)

HIV: human immunodeficiency virus

HMDS: hexamethyldisilazane

HMPA: hexamethylphosphoramide

HMPT: hexamethylphosphorous triamide

HMQC: heteronuclear multiple quantum correlation

Hz: Hertz

Ib: *iso*-butyl

iPr: *iso*-propyl

J: coupling constant (NMR)

lit.: literature

m: multiplet (NMR)

m-: meta

mCPBA: *meta*-chloroperbenzoic acid

Me: methyl

mg: milligram(s)

Mhz: megaHertz

min.: minute(s)

mmol: millimole(s)

mol: mole(s)

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

o-: ortho

p-: para

P: pressure

PG: protective group

Ph: phenyl

piv: pivaloyl

ppm: parts per million

psi: pounds per square inch (1 psi = 0.06804 atm)

q: quartet (NMR)

R_f: retardation factor

Rfx: reflux

RNA: ribonucleic acid

RT: room temperature

s: singlet (NMR)

sec.: second(s)

t: triplet (NMR)

T: thymidine

TBAF: tetrabutylammonium fluoride

tBDMS: *tert*-butyldimethylsilyl

tBDPS: *tert*-butyldiphenylsilyl

tBu: *ter*-butyl

TEA: triethylamine

TFA: trifluoroacetic acid

Th: thymine

THF: tetrahydrofuran

TLC: thin layer chromatography

TMS: trimethylsilyl

Tr: trityl (triphenylmethyl)

tRNA: *transfer* ribonucleic acid

Ts: tosyl (*para*-toluenesulfonyl)

U: uracil

v: volume

w: weight

Table of contents

"...science seldom proceeds in the straightforward logical manner imagined by outsiders. Instead, its steps forward (and sometimes backward) are often very human events in which personalities and cultural traditions play major roles"

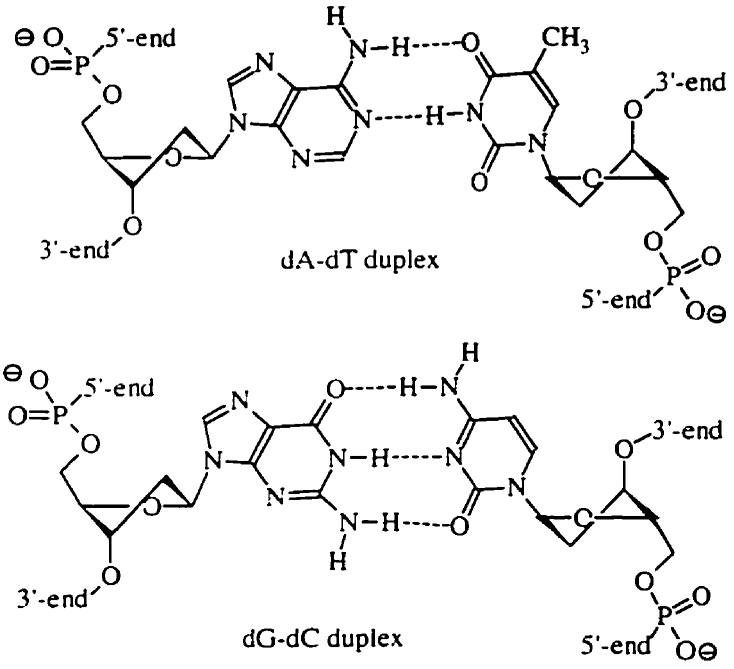
James D. Watson, *The Double Helix* **1968**

1. Introduction and literature survey

1.1. The antisense strategy

1.1.1. Principle of the approach

Observed for the first time in 1978 by Zamecnik and Stephenson[1,2], the antisense strategy became a new area of interest as a possible therapeutic method. Contrary to most therapeutic approaches, which proceed by trial-and-error and are consequently very demanding in terms of time, resources and cost, the antisense strategy was considered as a potential route to rational drug design. The principle is based on the complementarity, via hydrogen bonds, of purine and pyrimidine bases first proposed by Watson and Crick[3] as shown in scheme 1.1. On one hand, adenine recognizes specifically thymine, on the other



Scheme 1.1: the Watson-Crick base pairing

hand guanine recognizes specifically cytosine. This complementarity allows DNA, by varying the sequence in which these four bases are used, to store the fantastic database

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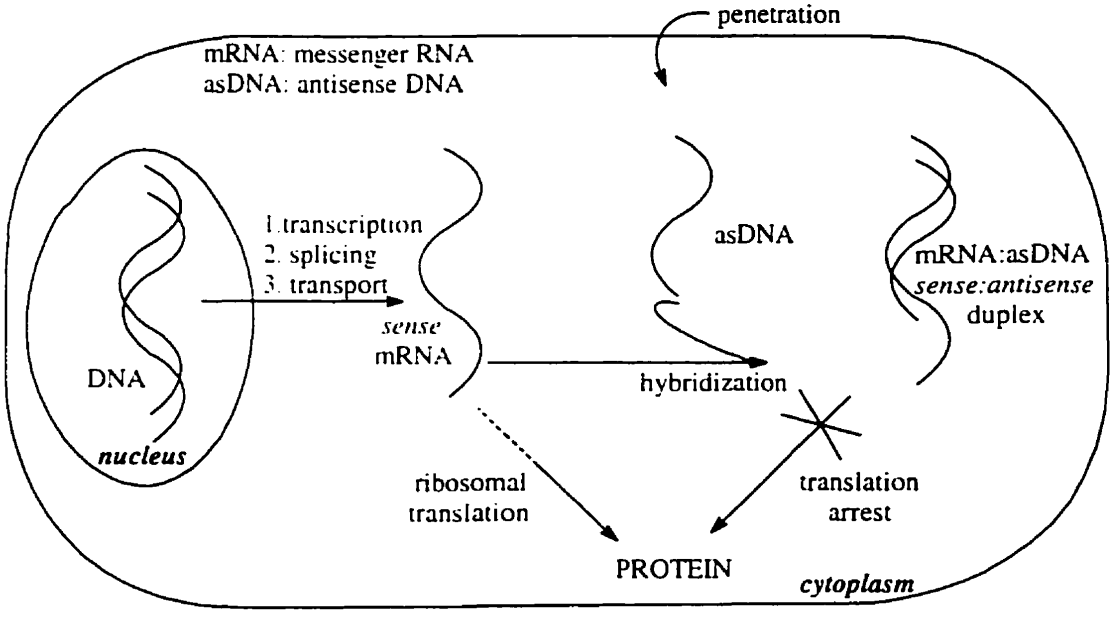
[1] Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 280
[2] Stephenson, M. L.; Zamecnik, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 285
[3] Watson, J. D.; Crick, F. H. *Nature* **1953**, *171*, 737

containing all the information necessary for a living cell and an organism to live and develop. By analogy to the alphabet, we may say that with only these four letters, nature is able to encode the principles of life and evolution.

This complementarity between DNA bases also constitutes one of the major stabilizing interactions of the antiparallel DNA double helix[4]. The second major interaction is electrostatic and resides in the stacking of the coplanar bases along the vertical axis of the double helix. These two interactions, along with steric, electrostatic and hydrophobic interactions, allow the DNA molecule to reach a very high stability, necessary to sustain and reproduce life.

The antisense strategy targets the messenger RNA (mRNA) issued from a gene after transcription. This mRNA conveys the information encoded in the DNA sequence, it will consequently later be called the "sense" strand. The aim is to introduce, into a living cell, an oligonucleotide, which will be called an "antisense" oligonucleotide, complementary in sequence to this mRNA in order to form an "antisense:sense" duplex that will prevent the genetic information from being conveyed further as indicated in



Scheme 1.2: role of the antisense oligonucleotide

[4] Saenger, W., "*Principles of Nucleic Acid Structure*", **1984**, Springer-Verlag Eds., pp 132-140

scheme 1.2. As opposed to most drugs that target DNA and are not or poorly sequence-specific such as adriamycin, bleomycin or cisplatin[5], the antisense DNA makes use of the huge amount of information contained in DNA.

Two mechanisms can be envisioned in order to keep the information from pursuing its usual road. The first one is the formation of a stable duplex between the antisense oligonucleotide and the mRNA that would simply keep the ribosome from attaching to the mRNA at the target sequence, therefore inhibiting the translation from a nucleotidic sequence into a peptidic sequence. The second, more widely accepted, is to activate the *RNAse H* enzyme that will degrade the mRNA strand of a hybrid DNA:RNA duplex[6]. With respect to the present goal, the result is the same, the translation from a nucleic acids alphabet into an amino acids alphabet cannot occur, therefore preventing the elaboration of a protein from the information conveyed by the mRNA. In principle, this translation arrest can occur at different stages during the life of the mRNA. One may reasonably think that the hybridization of the mRNA with the antisense oligonucleotide could first occur in the nucleus, before the transcription from DNA to mRNA is finished. It may also happen during the splicing of mRNA, or when the mRNA travels from the nucleus to the cytoplasm. Finally, as represented in scheme 1.2, it may happen when the mRNA is mature and ready to be translated into a protein by a ribosome. At the present time, the exact location of the action of antisense oligonucleotides within the cell remains to be elucidated.

Like other drugs, the antisense DNA has to meet several requirements in order to become a potential therapeutic agent. It has to be able to penetrate into a living cell, to be resistant to intracellular and extracellular enzymes. Otherwise, it would be degraded before having time to perform its therapeutic activity. It also has some requirements more particular to its own nature. It has to be specific to the target sequence, in order to inhibit only the information it is meant to stop, and not inhibit the translation of a gene encoded by another mRNA. In that aspect, its major asset is to use the same alphabet as that of DNA, which provides it with the required sequence specificity. Consequently, the

---

[5] Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543

[6] 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 298-299

sequence of the antisense DNA has to be longer than 16 units in order to statistically be able to inhibit the expression of a single gene among the entire human genome[7]. The antisense DNA also has to possess a good affinity for RNA, since it is designed to form a duplex with the mRNA. Finally, it has to be available at a reasonable cost.

As mentioned earlier, Zamecnik and Stephenson were the first to use the antisense strategy to inhibit gene expression. They inhibited the growth of the *Rous sarcoma* virus in cell culture with a 13-mer DNA oligonucleotide made of natural phosphodiester bonds[1].

However, a DNA oligonucleotide made of natural phosphodiester linkages alone could not be used efficiently for the *in vivo* inhibition of gene expression because it did not satisfy two of the criteria enumerated above. First, being a highly polar molecule it was initially thought not to be able to cross easily the hydrophobic phospholipid bilayer constituting the cell membrane and therefore could not efficiently penetrate the intracellular medium. Second, once inside the cell it was not stable to nucleases and was degraded too quickly. These two limitations greatly reduced the potential of regular DNA as an antisense agent, and opened a new area of research for organic chemists: the synthesis of antisense oligonucleotides, analogues of DNA.

1.1.2. DNA analogues as antisense agents

The DNA analogues designed by chemists to play a role as antisense agents had to meet the same requirements as the ones given in section 1.1.1. The DNA molecule, by its chemical complexity, lent itself to a large variety of possible modifications[8]. These can be divided into three main categories: the modifications to the base, to the sugar and to the backbone of the DNA molecule, as illustrated in scheme 1.3.

Modifications to the base[9] have a rather limited scope since one wants to maintain the Watson-Crick base pairing that gives the antisense its specificity to the target sequence. However, a number of research groups have attempted this class of modifications, with various degrees of success. For example, the use of 5-methyl or 5-

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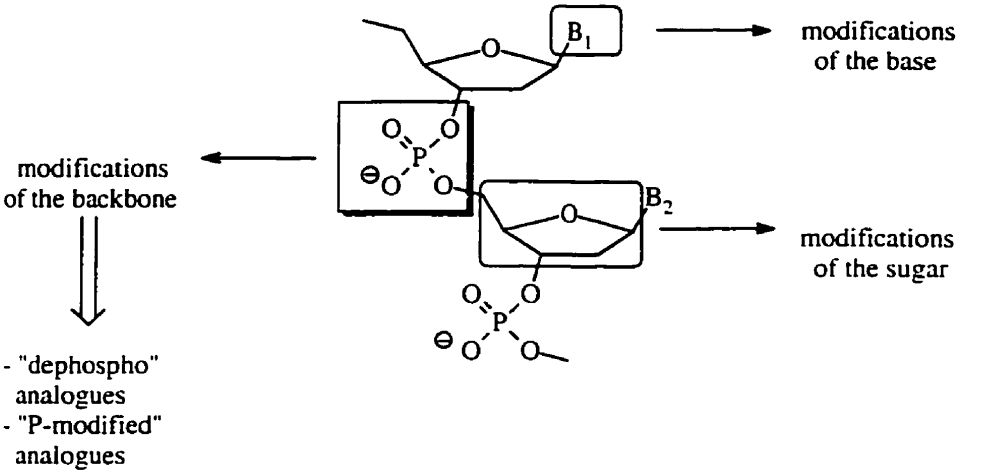
[7] Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923

[8] Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543

[9] *for a review, see*: Shangvi, Y. S. in "*Antisense Research and Applications*"; Crooke, S. T.; Lebleu, B., Eds.; CRC Press, Inc.; **1993**, pp 273-88

bromocytosine[10], 5- ethynyluridine[11], 5-(1-propynyl)-2'dU or dC[12], pseudouridine[13], 2-



Scheme 1.3: possible sites of modifications of DNA

aminoadenine[14] enhanced the binding to complementary RNA. In some cases, they had to be used in conjunction with backbone modifications since some of these base alterations did not give the antisense oligonucleotide the necessary resistance to nucleases.

Modifications to the sugar have also been exploited[15]. For example, α-anomeric nucleosides having a parallel orientation to the target strand were shown to have an increased duplex stability[16] and a stability to nucleases[17]. Some hexapyranosyl nucleotides also exhibited an increased duplex stability[18]. The introduction of 2'-fluoro[19] or 2'-O-

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[10] Shangvi, Y.; Hoke, G. D.; Freier, S. M.; Zounes, M. C.; Gonzalez, C.; Cummins, L.; Sasmor, H.; Cook, P. D. *Nucleic Acids Res.* **1993**, *21*, 3197

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[15] *for a review, see* : De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366 *and references cited therein*

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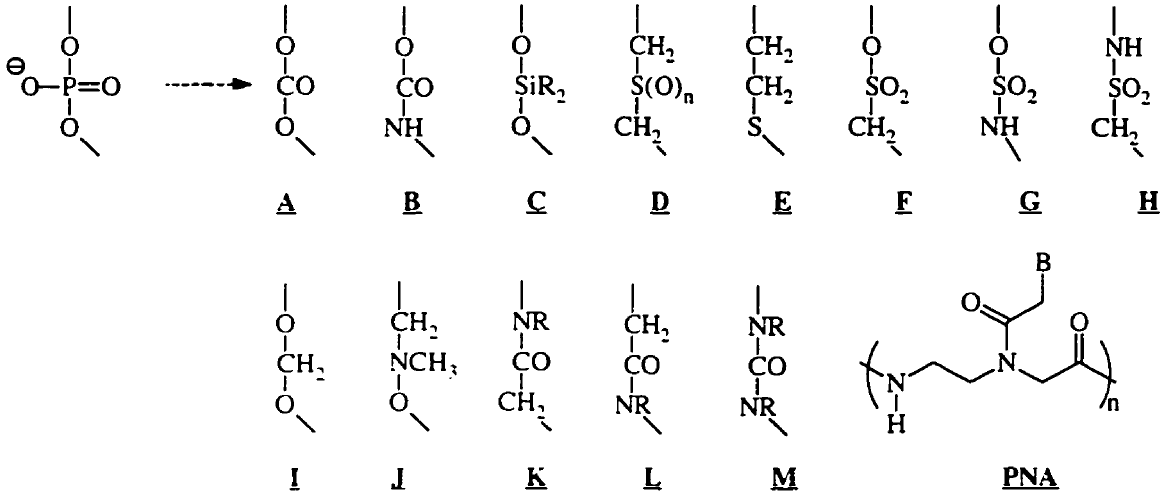
[18] Herdewijn, P.; De Winter, H.; Doboszewski, B.; Verheggen, I.; Augustyns, K.; Hendrix, C.; Saison-Behmoaras, T.; De Ranter, C.; Van Aerschot, A. in "*Carbohydrate Modifications in Antisense Research*"; ACS Symposium Series 580; Sanghvi, Y. S.; Cook, P. D., Eds; American Chemical Society; Washington, D. C. **1994**; pp 80-99

[19] 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

methyl[20,21] substituents increased the affinity towards RNA by orienting the sugar in the more *RNA-like* 3'-*endo* conformation but decreased the duplex stability. Incorporation of 2'-O-allyl substituents also increased the stability of duplexes with RNA[15].

Until now, 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. Two categories could be defined: the ones replacing the phosphorus atom by another functionality, and the ones keeping this atom with modifications around it. For the first "dephospho" category, many alterations are possible around the four squelettal atoms that constitute the internucleosidic bridge. As indicated in scheme 1.4, oligonucleotides were



Scheme 1.4: several "dephospho" analogues

synthesized, containing one or several carbonate[22,23] **A**, carbamate[24,25] **B**, dialkyl or diarylsilyl[26,27] **C**, thioethers[28] **D** (n=0) and **E**, sulfoxide **D** (n=1), sulfone[29,30] **D** (n=2),

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[20] Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131
[21] 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
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sulfonate[31] **F**, sulfonamides[32] **G** and **H**, formacetal[33,34] **I**, methylene(methylimino) (MMI)[35] **J**, amides[36,37] **K** and **L**, ureas[38] **M** internucleosidic bridges. Their hybridization properties with RNA or DNA were subsequently tested. Some showed better binding to RNA than phosphodiester DNA, some were worse, all of them displayed an increased resistance to nucleases when tested. However, the synthesis of these "dephospho" analogues often involved a large number of steps and was not applicable to solid phase synthesis, making them impractical for large scale synthesis. The synthesis of MMI analogue **J** was recently achieved through solid phase synthesis[39]. To be noted, Benner and co-workers[40] reported recently the first synthesis of a fully nonionic RNA analog, consisting in an RNA octamer incorporating dimethyl sulfone linkages only. Such synthetic oligonucleotides are extremely useful tools not only as antisense agents, but also as probes of the structures of DNA, RNA, their interactions with antisense strands as well as their physico-chemical properties. The peptide nucleic acids (PNAs) have drawn a lot of attention recently, since they have been shown to bind more strongly than natural DNA[41]. They involve the replacement of both the sugar and the DNA backbone by an amide backbone.

The other option to modify the backbone of DNA was to keep the phosphorus atom, and change the substituents around it, giving what will be called the "P-modified" oligonucleotides. As indicated in scheme 1.5, phosphorothioates[42] **N**, methylphosphonates[43] **O**, phosphoramidates[44,45] **P**, phosphotriesters[46] **Q**, phosphorodithioates[47] **R**,

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[31] Musicki, B.; Widlanski, T. S. *J. Org. Chem.* **1990**, *55*, 4231
[32] Huie, A. M.; Kirshenbaum, M. R.; Trainor, G. L. *J. Org. Chem.* **1992**, *57*, 4569
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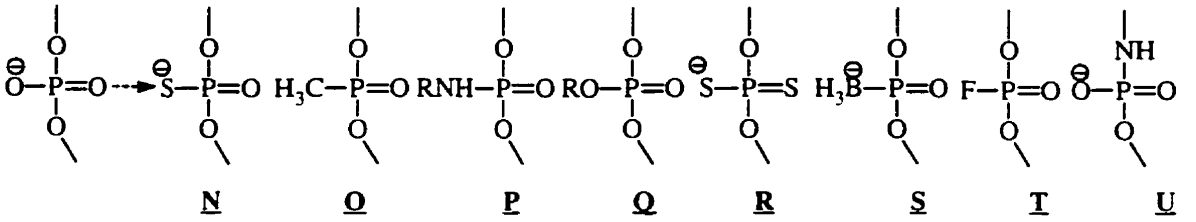
boranophosphates[48] **S** and phosphorofluoridites[49] **T** have all been synthesized as possible



Scheme 1.5: "P-modified" DNA analogues

antisense agents. One of the remarkable modifications has been the introduction of phosphoramidates **U** by Gryaznov et al.[49b], which showed enhanced binding to RNA. The most studied DNA analogues of these class have been the phosphorothioates, and to a lesser extent the methylphosphonate derivatives.

1.2. DNA phosphorothioates

As mentioned in section 1.1, DNA phosphorothioates have been the most extensively studied antisense oligonucleotides so far. They also are the most advanced antisense agents since they have reached different levels of clinical studies against various diseases.

1.2.1. Applications

First, the nuclease stability of oligonucleotides is increased when phosphodiester internucleotidic bridges are replaced by phosphorothioate bridges. Phosphorothioate oligonucleotides are stable in cells, cell extracts, serum, various tissues, urine and they display an increased stability to most nucleases[50,51,52]. The half-life of a pentadecamer oligonucleotide made of phosphorothioate bridges has been found to be >24 h in serum,

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[49] Dabkowski, W.; Cramer, F.; Michalski, J. *Tetrahedron Lett.* **1987**, *28*, 3561
[49b] Gryaznov, S.; Chen, J.-K. *J. Am. Chem. Soc.* **1994**, *116*, 3143
[50] Campbell, J. M.; Bacon, T. A.; Wickstrom, E. *J. Biochem. Biophys. Methods* **1990**, *20*, 259
[51] Stein, C. A.; Cohen, J. S. *Cancer Res.* **1988**, *48*, 2659
[52] Crooke, S. T.. *Annu. Rev. Pharmacol Toxicol.* **1992**, *32*, 329

whereas the same sequence phosphodiester oligonucleotide has a half-life of about 1h[52]. Furthermore, phosphorothioate oligonucleotides are more stable to various restriction endonucleases when in duplexes. In general, there is a significant difference in the rate of cleavage of the (R_p) and (S_p) phosphorothioate linkages[53,54,55,56].

Like phosphodiesters, phosphorothioate oligonucleotides are highly charged, polyanionic molecules. Because of the replacement of oxygen by sulfur atoms, they are more lipophilic than phosphodiesters. Contrarily to the widely accepted belief that highly charged DNA molecules cannot be internalized by cells, several groups have shown that DNA molecules, of both low and high molecular weights, can be internalized by cells[57,58,59,60]. Studies have shown that phosphorothioate oligonucleotides are also internalized by cells, in a mechanism that remains controversial[61]. For example, uptake of a 21-mer phosphorothioate oligonucleotide uniformly labeled with ^{35}S was characterized in *HeLa* cells[52]. The uptake is temperature, sequence, length and cell-dependent. The fact that this uptake is energy- and temperature-dependent[62,63] indicates that the process is active and that oligonucleotide phosphorothioates do not passively diffuse through the cell membrane. Beltinger *et al.* have recently demonstrated that the mechanism of uptake of phosphorothioate oligonucleotides is dependent upon the concentration of the oligonucleotide[64]. They also have shown that phosphorothioate oligonucleotides travel via the endosomal-lysosomal pathway once inside the cell. These oligonucleotides have been

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[54] Eckstein, F. *Annu. Rev. Biochem.* **1985**, *54*, 367
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[58] Loke, S. L.; Stein, C. A.; Zhang, X. A.; Mori, K.; Nakanishi, M.; Subashinge, C.; Cohen, J. S.; Neckers, L. M. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 3474
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[60] Stein, C. A.; Tonkinson, J. L.; Zhang, L.-M.; Yakubov, L.; Gervasoni, J.; Taub, R.; Rotenberg, S. A. *Biochemistry* **1993**, *32*, 4855
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found to accumulate in vesicular structures and in the nucleus, significantly less in the cytoplasm. This may mean that the oligonucleotides are first trapped into vesicles, and that they are subsequently released directly from the vesicles into the nucleus. This observation may imply that the antisense action of these oligonucleotides takes place into the nucleus. One may consequently consider that they act through the formation of mRNA:DNA duplexes or by interfering at the level of transcription, processing of mRNA (splicing), or transport towards the cytoplasm.

In terms of applications as antisense agents, phosphorothioate oligonucleotides have proven to be extremely useful tools. By substituting one or several phosphodiesters by a phosphorothioate bridge on a sequence usually cleaved by the restriction endonuclease *Eco*RV, Eckstein and co-workers were able to map the hydrolytic activity of that enzyme[65]. Using diastereomerically pure phosphorothioate bridges incorporated into a phosphodiester oligonucleotide, Koziolkiewicz and Stec have studied the stereochemical aspects of DNA-*Eco*RI endonuclease interactions[66]. Phosphorothioate oligonucleotides have been used as antisense agents by different groups, in order to inhibit the expression of various genes. Cohen and co-workers[67] have shown that phosphorothioate oligonucleotides were sequence-specific and more potent inhibitors of the leukemic cell growth and survival than phosphodiesters, when the human BCL2 protooncogene was targeted. Sakakura *et al.*[68] have demonstrated it was possible to inhibit the *in vitro* proliferation of colon cancer cells by targeting the Ki-ras protooncogene with phosphorothioate oligonucleotides.

However, in some cases phosphorothioate oligonucleotides displayed an *in vitro* inhibition that was not sequence specific. For example, Ho *et al.*[69] showed that a phosphorothioate oligomer complementary to the human transferrin receptor gene could inhibit the expression of this receptor in HL-60 leukemia cells, whereas its equivalent

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[65] Olsen, D. B.; Kotzorek, G., Eckstein, F. *Biochemistry* **1990**, *29*, 9546
[66] Koziolkiewicz, M.; Stec, W. J. *Biochemistry* **1992**, *31*, 9460
[67] Reed, J. C.; Stein, C.; Subasinghe, C.; Haldar, S.; Croce, C. M.; Yum, S.; Cohen, J. S. *Cancer Res.* **1990**, *50*, 6565
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[69] Ho, P. T. C.; Ishiguro, K.; Wickstrom, E.; Sartorelli, A. C. *Antisense Res. Dev.* **1991**, *1*, 329

phosphodiester did not. They also observed that the degree of receptor inhibition was not sequence-dependent. Several explanations can be put forward to account for this observation. According to the first one, this non-sequence specificity would come directly from the stereochemistry at the phosphorus center. One diastereomer would allow a tight binding to the target, whereas the other would not and therefore allow for binding to other sequences due to a low discrimination of the sequence of bases. The second explanation would be that one diastereomer is hydrolyzed whereas the other one is not. Consequently, this hydrolysis would give rise to smaller fragments which would not have the minimal length necessary to inhibit a single target sequence. Therefore, these smaller fragments could find several targets that have the complementary sequence and provoke an undesired effect.

Pyrimidine phosphorothioate oligonucleotides have also been found to have the capacity to form triple-stranded helices when targeted at a DNA homopurine:homopyrimidine duplex[70], therefore making them potential candidates for the antigene strategy[71] when double-stranded DNA is used directly as a target in order to form a triple helical structure. They have recently been shown to be able to inhibit restriction enzyme Ksp 632-I via triple helix formation[72].

The uses and applications of phosphorothioate oligonucleotides for various biological roles have been the subject of numerous studies, making them the most widely used antisense agents up to this time[73].

Perhaps the most promising potential associated to DNA phosphorothioates as antisense agents remains in the fact that phosphorothioates have been reported as inhibitors of the human immunodeficiency virus (HIV). In 1988, Zamecnik and co-workers[74] showed that several oligonucleotides, including phosphorothioates, could inhibit the replication of the HIV *in vitro*. They later demonstrated, in agreement with Matsukura

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[70] Xodo, L.; Alunni-Fabbroni, M.; Manzini, G.; Quadrifoglio, F. *Nucleic Acids Res.* **1994**, *22*, 3322

[71] Thuong, N. T.; Hélène, C. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 666

[72] Tsukahara, S.; Yamakawa, H.; Takai, K.; Takaku, H. *Nucleosides Nucleotides* **1994**, *13*, 1617

[73] for a review on applications, see: Zon, G.; Stec, W. J. "*Phosphorothioate Oligonucleotides*", in "*Oligonucleotides and Analogues, a Practical Approach*" **1991**, F. Eckstein Ed., The Practical Approach Series, IRL Press, pp. 87-108

[74] Agrawal, S.; Goodchild, J.; Civeira, M. P.; Thornton, A. H.; Sarin, P. M.; Zamecnik, P. C. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 7079

et al.[75], that phosphorothioate oligomers could sequence-specifically inhibit the replication of that same virus in early infected and chronically infected cells up to 100 times more potently than their corresponding phosphodiester oligonucleotides[76].

1.2.2. The structure of DNA phosphorothioates

As is now clear, phosphorothioate oligonucleotides represent an important class of antisense oligonucleotides. However, they possess some deficiencies that ought to be overcome.

From a structural point of view, the modification is extremely simple, and involves the replacement of one of the non-bridging oxygen atoms by the next chalcogen element in the periodic table: sulfur.

Perhaps their major weakness is their stereochemical irregularity. The phosphorothioate linkage introduces an additional stereogenic center within the DNA molecule. By replacing one of the prochiral non-bridging oxygen atoms of the achiral phosphodiester linkage by a sulfur atom, a new center of chirality is generated (scheme 1.6). The absolute stereochemistry at the phosphorus atom is specified as (S_p) or (R_p). Therefore if a phosphorothioate dimer is synthesized with no control of the stereochemistry at the newly generated internucleotidic bridge, two diastereomers are thus obtained in a ratio close to 1:1. Actually, this ratio is not exactly 1:1 since both nucleosides are chiral, thus influencing the chirality of the phosphorothioate linkage[77,78]. As discussed in section 1.3, DNA phosphorothioates are now easily obtainable from solid phase synthesis, using the phosphoramidite methodology or the H-phosphonate approach. The only step that differs from the usual synthesis of phosphodiester oligomers is the oxidation step, which has to be replaced by a sulfuration step. Different sulfurizing reagents have been developed and assessed[79,80,81,82,83]. This sulfurization step has proven to be

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[75] Matsukura, M.; Zon, G.; Shinozuka, K.; Robert-Guroff, M.; Shimada, T.; Stein, C. A.; Mitsuya, H.; Wong-Staal, F.; Vohen, J. S.; Broder, S. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 4244

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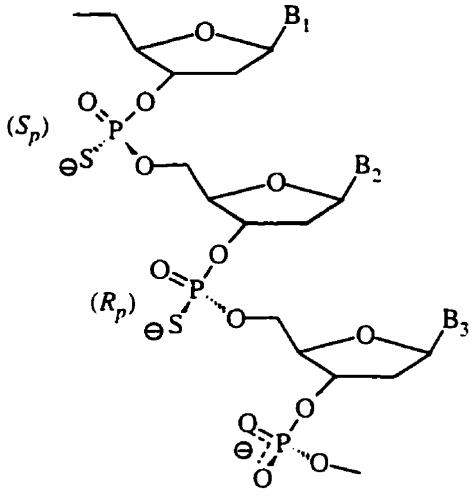
stereospecific[84,85,86,87] with retention of configuration when applied to phosphite



B_1, B_2, B_3 = Ad, Th, Cy, Gu

Scheme 1.6: stereochemistry of the phosphorothioate linkage

triesters or to H-phosphonates, providing access to phosphorothioate triesters or diesters respectively in very high yields.

Two diastereomers are characterized by different physico-chemical properties[88], and consequently different biological properties. For example in the present case, oligonucleotide phosphorothioates possessing only (R_p) internucleotidic linkages were found to be resistant to endonuclease P1[89], whereas the (S_p) oligonucleotides were all cleaved under the same conditions. Contrarily to these results, *snake venom* phosphodiesterase digested only terminal nucleotides having the (R_p) configuration[90].

Considering the synthesis of an oligonucleotide of twenty units, the number of possible diastereomers would be 2^{19}, in other words more than five hundred thousand

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[80] Iyer, R. P.; Egan, W.; Ryan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253
[81] Vu, H.; Hirschbein, B. L. *Tetrahedron Lett.* **1991**, *32*, 3005
[82] Rao, M. V.; Reese, C. B.; Zhengyun, Z. *Tetrahedron Lett.* **1992**, *33*, 4839
[83] Efimov, V. A.; Kalinkina, A. L.; Chakhmakhcheva, O. G.; Schmaltz Hill, T.; Jayaraman, K. *Nucleic Acids Res.* **1995**, *23*, 4029
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[86] Stec, W. J.; Okruszek, A.; Michalski, J. *Angew. Chem. Int. Ed. Engl.* **1971**, *10*, 494
[87] Szafraniec, L. J.; Szafraniec, L. L.; Aaron, H. S. *J. Org. Chem.* **1982**, *47*, 1936
[88] J. March "*Advanced Organic Chemistry, 4th ed.*" **1992**, John Wiley and Sons eds., p.113
[89] Griffiths, A. D.; Potter, B. V. L.; Eperon, I. C. *Nucleic Acids Res.* **1987**, *15*, 4145
[90] Burgers, P. M. J.; Eckstein, F.; Hunneman, D. H. *J. Biol. Chem.* **1979**, *254*, 7

different molecules[91]. It would be reasonable to think that each diastereomer possesses very particular physico-chemical as well as biological properties, which differ from one diastereomer to the next. This oligomer would then represent a mixture of compounds, not necessarily identical from one batch to the next, all possessing different pharmacological behaviors.

Since molecular recognition in biological systems is often dependent upon the stereochemistry of the molecules involved[92], one may reasonably hypothesize that the recognition of the target mRNA by the oligonucleotide phosphorothioate, its putative receptor-mediated transport and its interactions with the intracellular proteins would also be dependent upon the stereochemistry of the phosphorothioate linkage. As a therapeutic agent, it is also reasonable to think that much larger amounts of the oligonucleotide would be required in order to create the desired effect, since different components of the mixture would elicit biological responses of different intensities. The other constituents of the mixture may have an altered or even a different biological effect.

It therefore appeared important, from a theoretical and a practical point of view, to develop a method allowing the diastereoselective synthesis of phosphorothioate oligomers. This would be of great interest, not only for the antisense strategy, but also as a tool to investigate the mechanism of action of different enzymes and to study the influence of the stereochemistry on the stability of duplexes. Finally, this also presented a synthetic challenge, since at the present time the methods of diastereoselective synthesis of DNA phosphorothioates are limited in scope, as we shall now discuss in part 1.3.

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[91] Stec, W. J.; Wilk, A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 709
[92] König, B. *J. Prakt. Chem.* **1995**, 339

1.3. Stereocontrolled synthesis of phosphorothioates

This problem has been addressed in various ways. The present section will overview the different methods of controlling the stereochemistry at phosphorus in the synthesis of DNA phosphorothioates.

First of all, the need for a stereocontrolled synthesis method became important as the methods of separation of these diastereomers were very limited. Practically, it was possible to separate diastereomeric dimers, difficult to separate trimers and almost impossible to separate tetramers. It is, at the present time, impossible to separate longer oligomers. This problem initially led to a block approach[93], whereby a dimer or trimer would be separated from a mixture of compounds synthesized by a non-stereocontrolled synthetic method, then coupled together, resulting in an oligomer containing only one internucleosidic linkage constituted by a mixture of diastereomers. The other bridges would have a known sense of stereochemistry.

1.3.1. Enzymatic synthesis

Nature is a master in the art of controlling the stereochemical outcome of biochemical processes. This ability has not been matched by chemists at this time, and it has inspired the use of natural tools in order to carry out stereocontrolled reactions. These tools are obviously enzymes, and they have been applied to the stereocontrolled synthesis of phosphorothioates. Polymerases, transferases and nucleases have the ability to assist in the synthesis or degradation of phosphorothioates in a stereo-defined manner.

The enzymatic synthesis of polyribonucleotides containing phosphorothioate linkages was first pioneered by Eckstein and co-workers[94]. They used DNA-dependent RNA polymerase from *E. coli* to synthesize a modified RNA copolymer in which adenine and uridine units would alternate and every other internucleotidic bridge would be a phosphorothioate. Using uridine 5'-O-(1-thiotriphosphate) and adenosine 5'-O-(1-

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[93] Zon, G. "*High-Performance Liquid Chromatography in Biotechnology*" **1990**, W. S. Hancock, Ed.; Wiley, New York

[94] Matzura, H.; Eckstein, F. *Eur. J. Biochem.* **1968**, *63*, 448

thiotriphosphate) as mixtures of diastereomers, a polyribonucleotide containing only phosphorothioate linkages was synthesized later[95]. Eckstein determined the stereochemical course of polymerization by *E. coli* polymerase using 5'-0-(1-thiotriphosphate) as a substrate. The enzyme substrate was the (S_p) epimer and an inversion of configuration at the phosphorus center was observed, resulting in a phosphorothioate linkage having the (R_p) configuration[96,97]. Since then, several polymerases have been found to be able to catalyze the stereospecific formation of phosphorothioate DNA oligomers having consistently the (R_p) configuration. DNA-dependent DNA polymerases from *E. coli*[98,99], Phage T4[100,101], Phage T7[102], *Micrococcus luteus*[103], from polynucleotide phosphorylase[104,105], tRNA nucleotidyl transferase[106], RNA ligase[107] and 2'-5'-oligoadenylate synthetase[108,109] were all found to be able to catalyze the formation of 3'-5' or 2'-5' internucleotidic phosphorothioate linkages in a diastereospecific manner, always leading to an (R_p) configuration.

The only way to obtain an (S_p) linkage using enzymes would be to synthesize an oligomer having random stereochemistry at the phosphorothioate linkage, and to subject the latter to a nuclease which would degrade the internucleotidic bridges having the (R_p) configuration, such as the ones discussed in section 1.2. For example from a tetramer, the result would be a mixture of tetramer, trimer and dimer, all having internucleotidic linkages

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[95] Eckstein, F.; Gindl, H. *Eur. J. Biochem.* **1970**, *13*, 558
[96] Eckstein, F.; Armstrong, V. W.; Sternbach, H. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73*, 2987
[97] Burgers, P. M. J.; Eckstein, F. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, *75*, 4798
[98] Burgers, P. M. J.; Eckstein, F. *J. Biol. Chem.* **1979**, *254*, 6889
[99] Brody, R. S.; Frey, P. A. *Biochemistry* **1981**, *20*, 1245
[100] Romaniuk, P. J.; Eckstein, F. *J. Biol. Chem.* **1982**, *257*, 7684
[101] Gupta, A.; DeBrosse, C.; Benkovic, S. J. *J. Biol. Chem.* **1982**, *257*, 7689
[102] Brody, R. S.; Adler, S.; Modrich, P.; Stec, W. J.; Lesnikowski, Z. J.; Frey, P. A. *Biochemistry* **1982**, *21*, 2570
[103] Eckstein, F.; Jovin, T. M. *Biochemistry* **1983**, *22*, 4546
[104] Burgers, P. M. J.; Eckstein, F. *Biochemistry* **1979**, *18*, 450
[105] Marlier, J. F.; Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 2212
[106] Eckstein, F.; Sternbach, H.; von der Haar, F. *Biochemistry* **1977**, *16*, 3429
[107] Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1982**, *21*, 5877
[108] Suhadolnik, R. J.; Choongeun, L. *Biochemistry* **1985**, *24*, 551
[109] 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

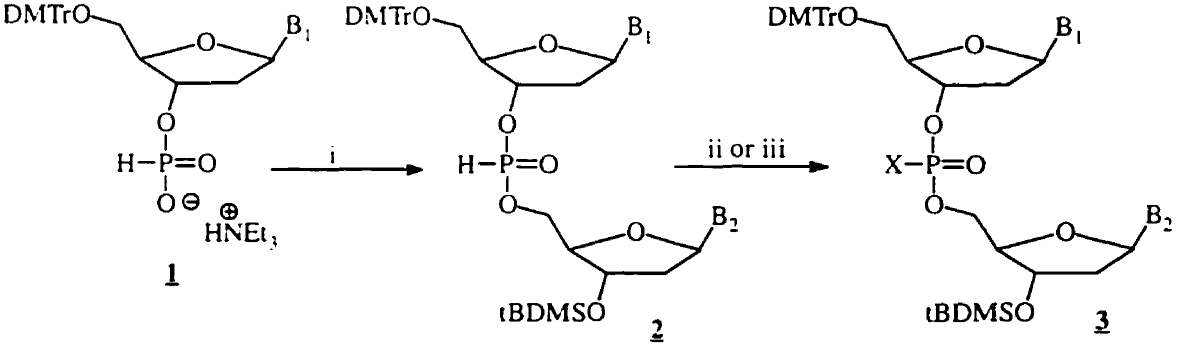
with (S_p) configuration. The yield would obviously be dramatically low, making this method highly impractical.

As a result, the need for an efficient, highly diastereoselective chemical method of synthesis of phosphorothioate dimers became an important issue. The approaches that have been tried by various groups will now be presented and discussed. Several methods were considered by different research groups around the world to achieve a stereocontrolled synthesis of DNA phosphorothioates, given the high therapeutic potential of these derivatives. In the following sections, these methods will be summarized, as well as their advantages and limitations.

Two types of reactions were used, namely the reaction via H-phosphonate precursors or the stereoselective reaction of tetravalent phosphorus derivatives. The first approach will now be described.

1.3.2. Synthesis of phosphorothioates from chiral H-phosphonates

In 1991, Seela and Kretschmer[110] described the synthesis, via H-phosphonate building blocks, of the following stereo-pure dinucleotides dA-dA, dC-dA, dG-dA, T-dA, dA-T, dC-T, dG-T, T-T, containing a methylphosphonate or phosphorothioate internucleotidic bridge (scheme 1.7). In this synthesis, the first step



i. 3'-O-tBDMS-nucleoside, tBuCOCl, C_5H_5N; ii. CH_3I / BuLi gives X = CH_3

iii. S_8 in CS_2 / C_5H_5N gives X = S

(B_1, B_2) = (Ad^{Bz}, Ad^{Bz}); (Cy^{Bz}, Ad^{Bz}); (Gu^{Ib}, Ad^{Bz}); (Th, Ad^{Bz}); (Ad^{Bz}, Th); (Cy^{Bz}, Th); (Gu^{Ib}, Th); (Th, Th)

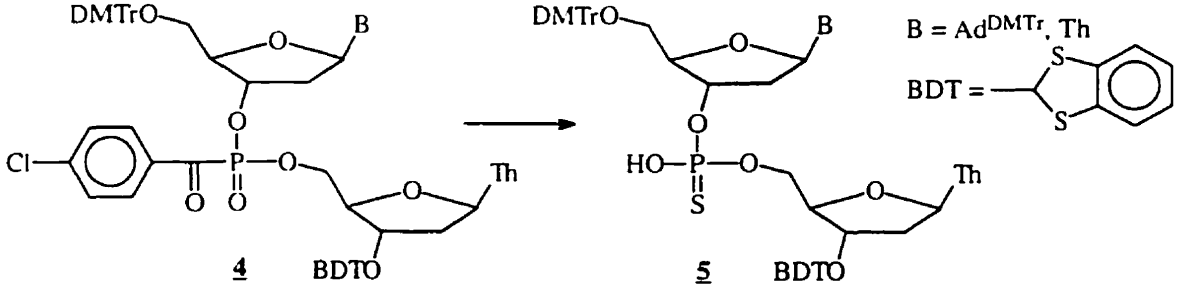
Scheme 1.7: synthesis of phosphorothioates according to Seela and Kretschmer

[110] Seela, F.; Kretschmer, U. *J. Org. Chem.* **1991**, *56*, 3861

involved the reaction of the triethylammonium salt of a 5'-O-DMTr-3'-H-phosphonate functionalized nucleoside **1** with a 3'-O-tBDMS-nucleoside (B_2 component), using pivaloyl chloride in pyridine, to give the two diastereomeric building blocks of H-phosphonate **2**, which were then separated chromatographically. Each pure diastereomer of the newly formed H-phosphonate **2** was then methylated (MeI/BuLi) or sulfurized ($S_8/CS_2/C_5H_5N$) to give the corresponding methylphosphonate or phosphorothioate **3** in a diastereospecific manner. Other examples of the use of diastereomerically pure H-phosphonates were reported, all involving the initial synthesis of both diastereomers of the intermediate H-phosphonate dimer, followed by chromatographic separation[111,112,113,114] and by a diastereoselective reaction.

To the best of our knowledge, there have been only two examples using an H-phosphonate approach in which the stereochemistry was generated at the phosphorus atom during the formation of the H-phosphonate moiety.

The first one was published by Hata and co-workers[115], who used acylphosphonate intermediate **4**, synthesized as a pair of diastereomers (scheme 1.8). With no separation of the two diastereomers, the *p*-chlorobenzoyl group on acylphosphonate **4** was removed in the presence of *n*-butylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and elemental sulfur to give exclusively the (R_p) isomer of phosphorothioate **5**. The two protective



Scheme 1.8: a fully stereoselective synthesis according to Hata and co-workers

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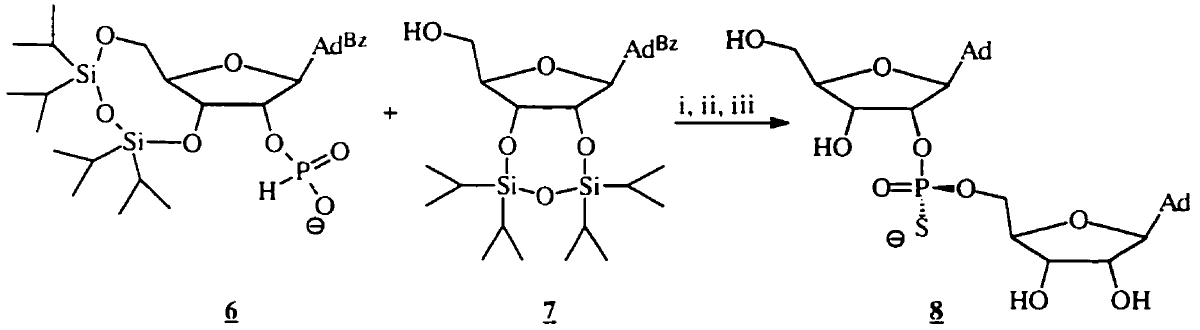
[111] Seela, F.; Kretschmer, U. *J. Chem. Soc., Chem. Commun.* **1990**, 1154
[112] Seela, F.; Kretschmer, U. *Nucleosides Nucleotides* **1991**, *10*, 711
[113] Stawinski, J.; Stromberg, R.; Zain, R. *Tetrahedron Lett.* **1992**, *33*, 3185
[114] Almer, H.; Stawinski, J.; Stromberg, R.; Thelin, M. *J. Org. Chem.* **1992**, *57*, 6163
[115] Fujii, M.; Ozaki, K.; Kume, A.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1986**, *26*, 935

groups were then removed with 0.5% TFA, producing the newly formed phosphorothioate dimer in 55-65% yield. Hata and co-workers[116] tried to elucidate the mechanism of this reaction, and noticed that when any of the two protective groups (DMTr or BDT) was replaced by another protection, the diastereoselectivity would drop dramatically. The effect was similar when the reaction was performed stepwise (initial removal of the aroyl group followed by sulfurization) or when any other pair of nucleosides was used. A mechanism involving a nucleophilic attack of DBU to form a bipyramidal pentacoordinated phosphorus intermediate was proposed by Hata and co-workers, but no such intermediate was isolated. In agreement with their proposal, Merckling and Rüedi[117] recently reported the isolation of a similar intermediate in another type of study.

The second example of this type of reaction was published by Battistini *et al.*[118]. As described in scheme 1.9, the authors reported the synthesis of dimers and trimers of 2',5'-oligo-(S_p)-thioriboadenylate upon condensation of 2'-H-phosphonate nucleoside **6** protected on the 3' and 5'-positions, with 2',3'-protected nucleoside **7** in the presence



6 **7** **8**

i. PivCl, C_5H_5N; ii. S_8; iii. deprotections (NH_4OH / C_5H_5N then TBAF / THF)

Scheme 1.9: diastereoselective synthesis of 2'-5'-phosphorothioates from Battistini *et al.*

of pivaloyl chloride in pyridine, followed by sulfurization. The synthesis of the dimer was again highly diastereoselective, and this diastereoselectivity was largely dependent upon the choice of protective groups. The authors rationalized the high diastereoselectivity in terms of the large steric hindrance created by the protective groups, preventing the reaction of the *pro-S* oxygen atom of H-phosphonate **6** with the bulky pivaloyl chloride,

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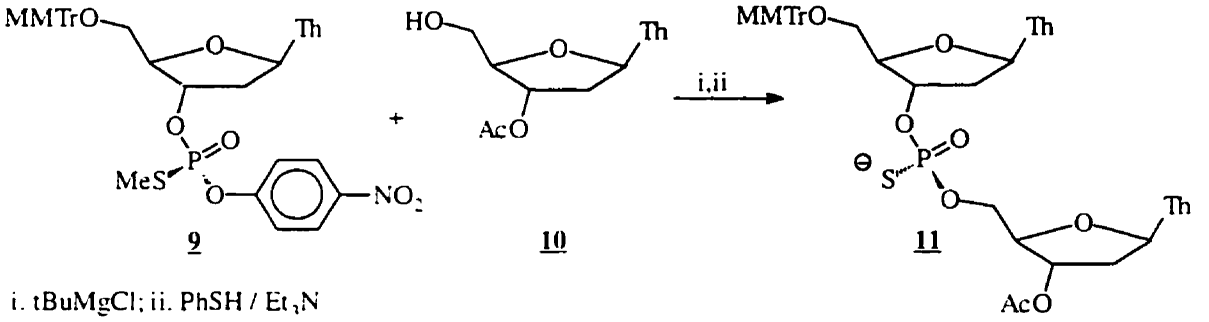
[116] Fujii, M.; Ozaki, K.; Sekine, M.; Hata, T. *Tetrahedron* **1987**, *43*, 3395
[117] Merckling, F. A.; Rüedi, P. *Tetrahedron Lett.* **1996**, *37*, 2217
[118] Battistini, C.; Brasca, M. G.; Fustinoni, S.; Lazzari, E. *Tetrahedron* **1992**, *48*, 3209

therefore directing the reaction to the *pro-R* oxygen atom and yielding only the (S_p) phosphorothioate dimer **8**. The synthesis of a trimer gave an (S_p)/(R_p) ratio of 80/20 at the second internucleosidic bridge.

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

1.3.3 Nucleophilic displacement at tetracoordinated phosphorus centers

Nucleophilic substitution at the phosphorus atom was the other alternative of choice to a stereoselective reaction at that center. Indeed, it was successfully used by several groups and is currently the most developed method of synthesis of phosphorothioate oligomers with a defined stereochemistry. Lesnikowski *et al.*[119,120,121,122] first reported the highly diastereoselective substitution of a *p*-nitrophenoxy group on nucleoside **9** by the 5'-hydroxyl group of nucleoside **10** at a tetracoordinated phosphorus center (scheme 1.10). The overall course of the reaction was an inversion of



Scheme 1.10: the stereospecific displacement of a *p*-nitrophenoxy group

configuration at the phosphorus atom, with a diastereoselectivity higher than 95% and an overall 70% yield in dimer **11**. Removal of the methyl group linked to the sulfur atom was subsequently performed with thiophenol and triethylamine. However, the starting activated triester **9** had to be prepared as a pair of diastereomers and then separated by

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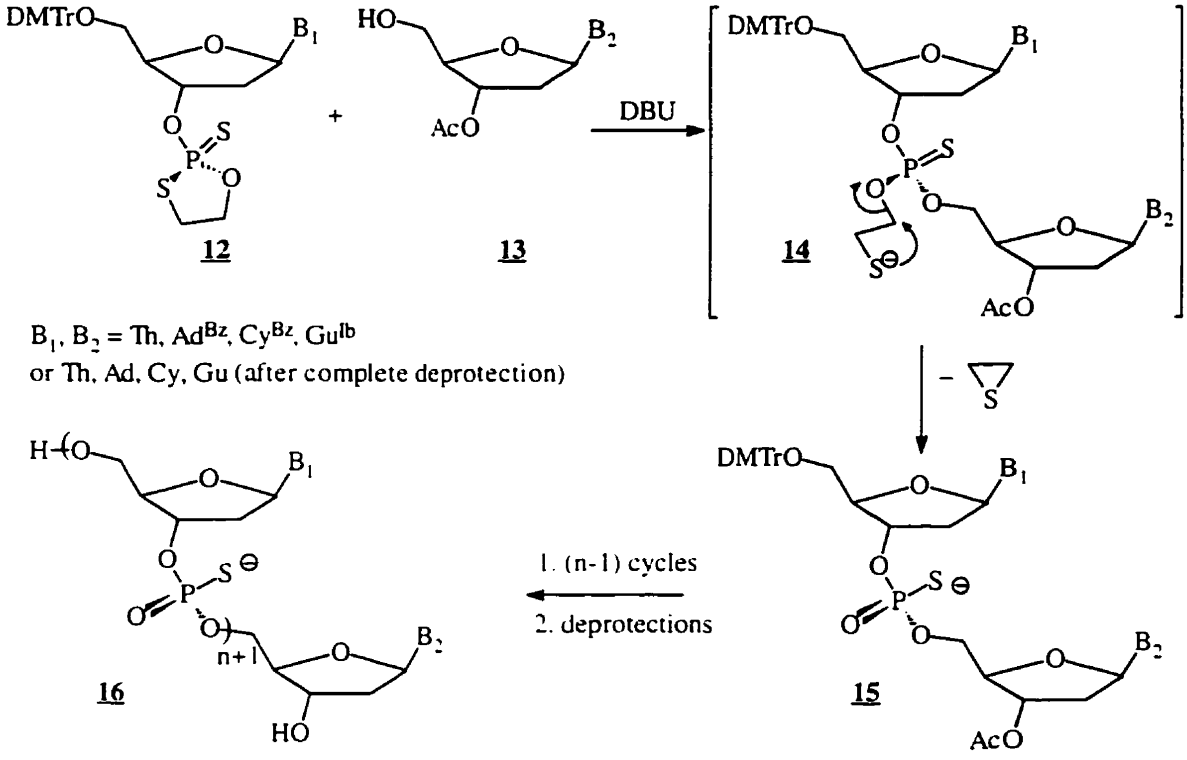
[119] Lesnikowski, Z. J.; Sibinska, A. *Tetrahedron* **1986**, *42*, 5025
[120] Lesnikowski, Z. J.; Jaworska, M. *Tetrahedron Lett.* **1989**, *30*, 3821
[121] Lesnikowski, Z. J. *Nucleosides Nucleotides* **1992**, *11*, 1621
[122] Lesnikowski, Z. J. *Bioorg. Chem.* **1993**, *21*, 127

chromatographic methods, which was probably the major drawback of this approach. The preparation of trimers and tetramers with a controlled stereochemistry was also performed by Lesnikowski *et al.*[20], with replacement of the methyl group by a 4-chlorobenzyl group.

Undoubtedly, the most advanced method of stereocontrolled synthesis of DNA phosphorothioates is the oxathiaphospholane method, developed by Stec and co-workers[123,124]. Similarly to the example quoted above, this method is based on a nucleophilic substitution at a tetracoordinated phosphorus atom as indicated in scheme1.11. In this approach, the starting oxathiaphospholane derivative **12** was first



Scheme 1.11: Stec's oxathiaphospholane method

prepared as a pair of diastereomers, then the two products were separated chromatographically. Subsequently, one of them was reacted with 3'-protected nucleoside **13** in the presence of a strong base (DBU), in order to perform the diastereospecific opening of the oxathiaphospholane ring to give intermediate **14**, immediately followed by

[123] Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, *19*, 5883
[124] 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

the spontaneous loss of episulfide. Phosphorothioate dimer **15** was then released, as a single diastereomer. When the 5'-free nucleoside (B_2 unit) was bound to a polymeric support, the cycle could be repeated and a longer oligomer **16** was thus obtained. Typically, the step-yield was around 94% and the diastereomeric excess d.e. >98%, for a coupling time of about 300 sec.

However, several weak points characterize this method. First, the chromatographic separation of the oxathiaphospholane diastereomeric mixture is very laborious and is accompanied by the loss of a large amount of precursor **12**. As reported by Stec *et al.*[124] : "For example, only 200 mg of "fast"-eluting and 150 mg of "slow"-eluting pure diastereomers were obtained from 1 g of the mixture after a 5-fold run of each partially enriched fraction through the column"[14]. Second, the condensation step leading from oxathiaphospholane **12** to phosphorothioate **15** requires the use of a 220-fold molar excess of DBU and a 20-fold molar excess of **12**.

At this point in time, the oxathiaphospholane method is the most advanced way to synthesize DNA phosphorothioates, and it has been applied to automated solid-phase synthesis after replacement of the succinyl linker by a succinylsarcosinyl linker resistant to DBU[125,126]. It allowed the authors to synthesize oligomers as long as pentadecamers (15 units) containing all four bases of DNA and with a known sense of stereochemistry[127] at each phosphorothioate linkage. Yet it is a method that is not applicable to large scale preparation, and clearly if DNA phosphorothioates with a defined stereochemistry are to be used in large quantities, a method that allows their synthesis on a large scale still remains to be developed and optimized.

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[125] Brown, T.; Pritchard, C. E.; Turner, G.; Salisbury, S. A. *J. Chem. Soc. Chem. Commun.* **1989**, 891

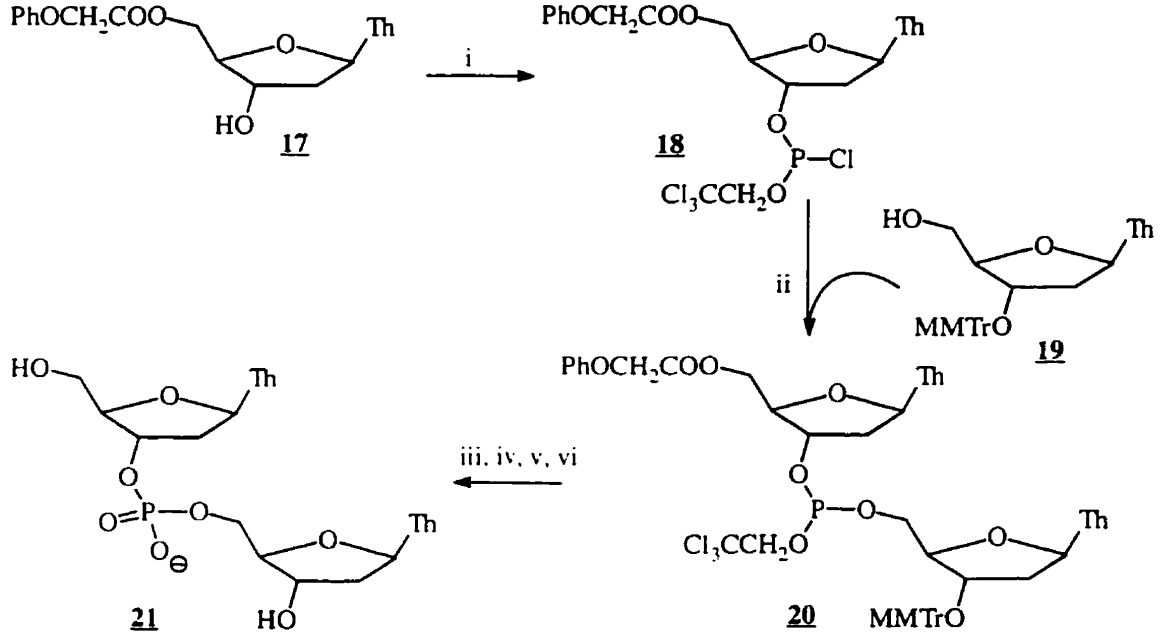
[126] Lehman, C.; Xu, Y.-Z.; Christoloulos, C.; Tan, Z. K.; Gait, M. J. *Nucleic Acids Res.* **1989**, *17*, 2379

[127] Koziolkiewicz, M.; Krakowiak, A.; Kwinkowski, M.; Boczkowska, M.; Stec, W. J. *Nucleic Acids Res.* **1995**, *23*, 5000

1.4. The phosphoramidite approach

1.4.1. Brief historical overview

The first chemical synthesis of a DNA dinucleotide via the H-phosphonate approach was reported in 1955 by Michelson and Todd[128]. Then, the use of diphenylphosphorochloridates was introduced by Todd and co-workers[129], followed by the use of phosphate monoesters by Letsinger and Ogilvie[130]. One of the limitations in these methods was the large amount of time required for each step, making them impractical for automation. Long reaction times allowed more side reactions to occur, therefore making the subsequent purification significantly more difficult. In 1975, Letsinger *et al.*[131,132] introduced phosphite triesters as precursors to phosphate triesters, as outlined in scheme 1.12. The great advantage of this method came from the higher reactivity of



i. $Cl_3CCH_2OPCl_2$, 2,6-lutidine, THF, -78°C; ii. **19**, 2,6-lutidine, THF; iii. I_2, H_2O; iv. NH_4OH, dioxane; v. CH_3COOH; vi. sodium naphthalene, HMPA then purification

Scheme 1.12: Letsinger's phosphite triester method

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[128] Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1955**, 2632
[129] Hall, R. H.; Todd, A.; Webb, R. F. *J. Chem. Soc.* **1957**, 3291
[130] Letsinger, R. L.; Ogilvie, K. K. *J. Am. Chem. Soc.* **1967**, *89*, 4801
[131] Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278
[132] Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655

trivalent phosphorus derivatives as opposed to their tetracoordinated analogues. The authors reported that phosphite triester dimers of nucleotides such as **20** could be synthesized very quickly from phosphorochloridites **18** and that they could in turn be oxidized rapidly to the corresponding phosphate triester. The phosphate triesters were then deprotected to give the phosphodiester units of DNA **21**.

The introduction of phosphite triesters laid the ground for the automation of oligonucleotide solid phase synthesis. After this major contribution, the use of solid supports was adapted to oligonucleotides synthesis by Letsinger and Mahadevan[133,134], following their use by Merrifield in peptide synthesis[135,136], and specific protective groups for the sugar[137] and base[138,139,140] components of nucleosides were developed to make the nucleosides compatible with highly reactive phosphorochloridite intermediates. All of these contributions allowed the solid phase synthesis of oligonucleotides to further develop, and to eventually become what is now a routinely used technique.

The initial synthesis of phosphite triesters has been modified since, and the phosphoramidite intermediates **23** first introduced by Beaucage and Caruthers[141] have become the precursors of choice (scheme 1.13[142]). They are less moisture sensitive and more easily handled than chlorophosphites introduced by Letsinger *et al.*[131]. The automated solid phase synthesis of DNA oligomers is now easily performed, the sequence and length of the fragments can be adjusted by the user. As an example, fragments as long as 175 units have recently been prepared[143] on a DNA synthesizer. The synthetic cycle typically takes about 8 min. per unit and the yield of each coupling step is >99%. After the

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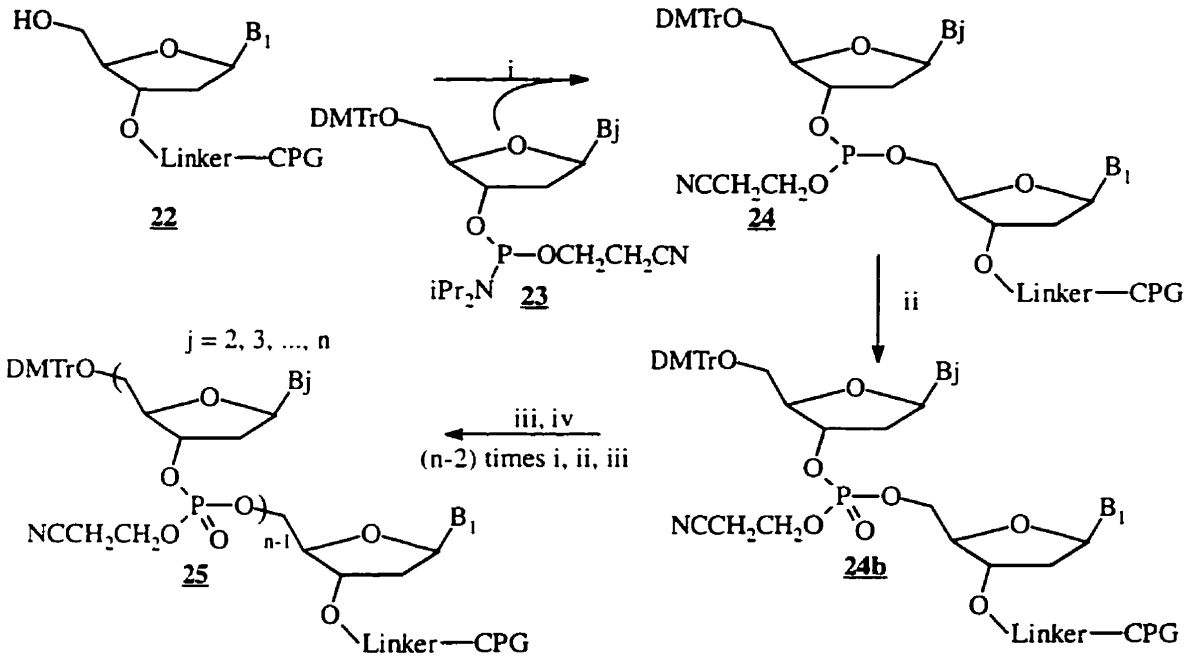
[133] Letsinger, R. L.; Mahadevan, V. *J. Am. Chem. Soc.* **1965**, *87*, 3526
[134] Letsinger, R. L.; Mahadevan, V. *J. Am. Chem. Soc.* **1966**, *88*, 5319
[135] Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149
[136] Merrifield, R. B. *Science* **1965**, *150*, 178
[137] Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *84*, 430
[138] Lohrman, R.; Soll, D.; Hayatsu, H.; Ohtsuka, E.; Khorana, H. G. *J. Am. Chem. Soc.* **1966**, *88*, 819
[139] Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316
[140] Agrawal, K. L.; Yamazak, A.; Cashion, P. J.; Khorana, H. G. *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 451
[141] Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859
[142] Brown, T.; Brown, D. J. S. "*Oligonucleotides and Analogues, a Practical Approach*" **1991**, Eckstein, F. ed., I. R. L. press, pp. 2-6
[143] Efcavitch, J. W.; McBride, L. J.; Eadie, J. S. *in Biophosphates and their Analogues/Synthesis, Structure, Metabolism and Activity*; Bruzik, K. S., Stec, W. J., Eds.; Elseviers Amsterdam. **1987**, p.205 quoted in Uhlmann, E.; Peyman, A. *Chemical Rev.***1990**, *90*, 543

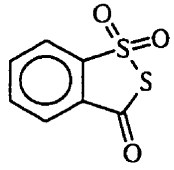
last cycle, a treatment with ammonium hydroxide allows for the removal of the protective



i. 23, tetrazole, CH_3CN; ii. I_2 / H_2O; iii. Ac_2O, N-Methyl imidazole (capping of the unreacted alcohols); iv. Cl_2CHCO_2H

Scheme 1.13: the phosphoramidite approach

groups from the bases, elimination of the cyanoethyl protective groups on the phosphate diester bridges, and cleavage of the newly formed polynucleotide from the solid support. This synthetic strategy was easily adapted to the synthesis of phosphorothioate derivatives or of mixed phosphate-phosphorothioates sequences by replacing all or some of the oxidative steps (iodine in water) by a sulfurization step, which could be performed using sulfurizing reagents, such as elemental sulfur S_8 or Beaucage's reagent[80] (3H-1,2-benzodithiol-3-one 1,1-dioxide, scheme 1.14).



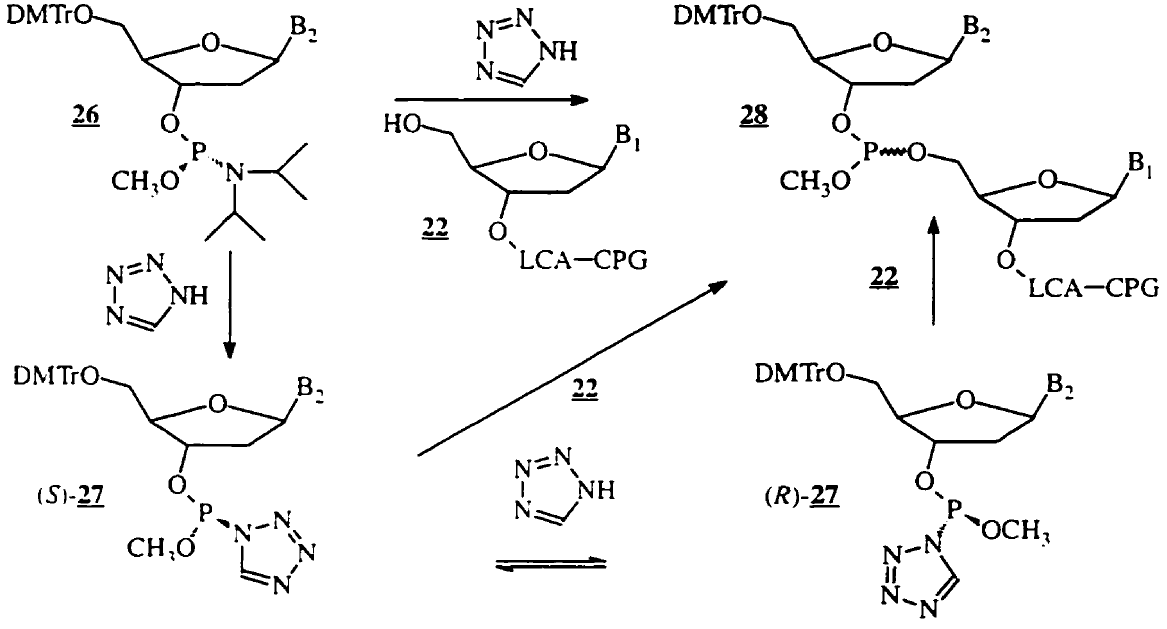
Scheme 1.14: Beaucage's sulfurizing reagent

However, in this case, the stereochemistry at the phosphorus atom was not controlled and the resulting product turned out to be a random mixture of diastereomers at

each phosphorothioate bridge. Actually, the chirality of the starting materials influenced the stereochemistry of the coupling step, so that each phosphorothioate bridge was not an equal mixture of both diastereomers.

1.4.2. The problem of epimerization

Such an elegant and efficient strategy was initially considered as a possible approach when researchers started to address the problem of stereochemistry in P-chiral DNA analogues. It seemed reasonable to separate the two diastereomers of phosphoramidite precursors **26** and to react a diastereomerically pure intermediate with a 3'-protected nucleoside in order to obtain a single diastereomer of the resulting phosphite triester **28** (scheme 1.13). This experiment was attempted by Stec and Zon[144] and resulted in an almost complete epimerization at the phosphorus atom. The mechanism of



Scheme 1.15: the mechanism of epimerization proposed by Stec and Zon

epimerization proposed by Stec and Zon is shown in scheme 1.15.

This mechanism was proven by Berner *et al.*[145,146] using ^{31}P NMR studies of the reactivity of phosphorotetrazolide derivatives analogous to (*R*)- and (*S*)-**27**. The activation

[144] Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279
[145] Berner, S.; Mühlegger, K.; Seliger, H. *Nucleosides Nucleotides* **1988**, *7*, 763
[146] Berner, S.; Mühlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853

by tetrazole involved, as expected, an initial protonation of the diisopropylamino function of the starting phosphoramidite. However, the amino group was not displaced directly by the alcohol group of the incoming nucleoside but by tetrazole, as observed by Dahl *et al.*[147]. A highly reactive phosphorotetrazolide (*S*)-**27** resulted, which epimerized quickly in the presence of tetrazole to its diastereomer (*R*)-**27** and eventually reacted with alcohol **22** in a slower process to yield the more stable and desired phosphite triester **28** as a mixture of two diastereomers. Consequently, the stereoselectivity was completely lost, constituting the reason why the phosphoramidite approach was abandoned early in the attempts to synthesize diastereomerically pure DNA phosphorothioates.

1.4.3. The use of heterocyclic precursors and modified catalysts

In order to adapt the phosphoramidite approach to a stereocontrolled synthesis of phosphite triesters, two essential requirements had to be met. The first one was the control of stereochemistry at the phosphorus center. Indeed, the classical phosphoramidite precursor (**23**, scheme 1.13) was generated as a pair of diastereomers which required a tedious chromatographic separation to be isolated as single diastereomers. The second one was the control of stereochemistry during the coupling step to yield the phosphite triester. Stec and Zon[144] proved that this step was not stereoselective because of the nucleophilic role played by tetrazole. Our laboratory began investigating this problem around 1991 and the early results will be presented in this section.

Xin and Just[148,149] met the first requirement (synthesizing a chirally pure phosphoramidite precursor) by incorporating the nitrogen, phosphorus and oxygen atoms in a six-membered oxazaphosphorinane ring derived from a chiral γ-aminoalcohol. They also addressed the problem of diastereoselectivity of the acid-catalyzed coupling step, and found that the classical catalysis by tetrazole was a limiting factor in this transformation. As indicated in scheme 1.16, chiral γ-aminoalcohol **29** was first synthesized and reacted with phosphorus trichloride and triethylamine to yield chiral oxazaphosphorinane **30**. The

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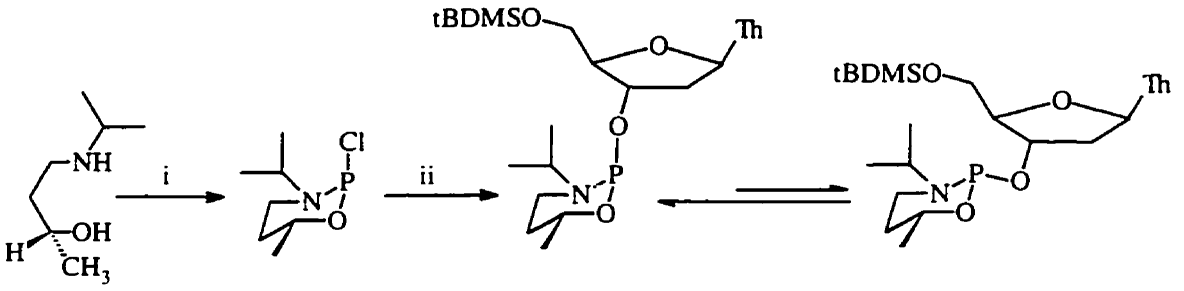
[147] Dahl, B. H.; Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 1729
[148] Xin, Z. *Master's Thesis*, McGill University, **1994**
[149] Xin, Z.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 969

reaction was followed by ^{31}P NMR and showed the presence of a single signal,



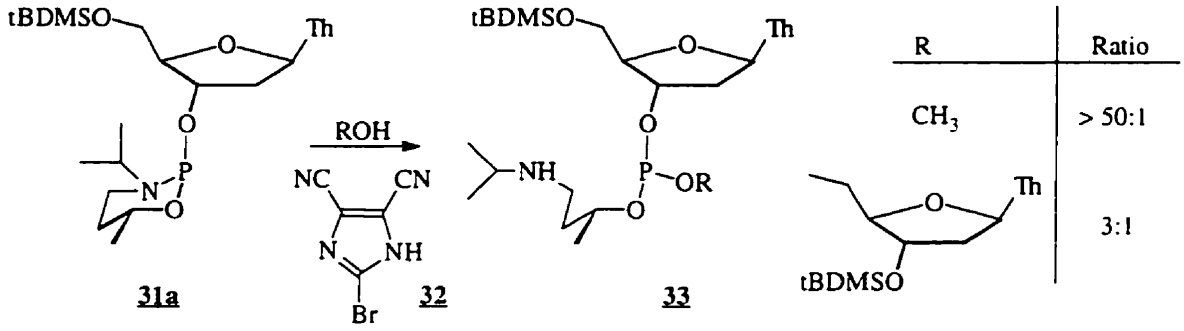
29 30 31a 31e

i. PCl_3, Et_3N, CH_2Cl_2; ii. 5'-O-tBDMS-thymidine, Et_3N, CH_2Cl_2, Rfx

Scheme 1.16: the first use of oxazaphosphorinane as a precursor

corresponding either to the more stable oxazaphosphorinane **30** bearing the chloro group in the axial position, or to the two epimers in fast equilibrium. With no isolation, this compound was reacted with 5'-O-tBDMS-thymidine and triethylamine to substitute the exocyclic chloro group by a protected nucleoside moiety. The reaction was followed by ^{31}P NMR and showed the initial formation at low temperature of two diastereomers **31a** and **31e** in a ratio of 1:3 respectively, which then evolved to a ratio of 20:1 upon reflux of the reaction mixture for several hours. After chromatography on a short column of silica gel, the major isomer **31a** was isolated as a pure diastereomer. This experiment proved that upon reaction with a chiral γ-aminoalcohol, diastereomerically enriched or pure phosphoramidite derivatives could be obtained, thus avoiding the very tedious chromatographic separation of an equimolar mixture of two diastereomers. When they performed the coupling step, Xin and Just first noticed that the reaction of phosphoramidite derivative **31a** with an alcohol and tetrazole as a catalyst, proceeded with a complete loss of stereoselectivity, which was in agreement with the mechanism proposed by Stec an Zon[144] outlined in scheme 1.15. As indicated in scheme 1.17, by varying the steric hindrance and the acidity of the catalyst, they managed to greatly increase the stereoselectivity of the coupling step in the case of a small alcohol like methanol. Ratios as high as 50:1 could be obtained when 2-bromo-4,5-dicyanoimidazole **32** was used as a catalyst. Reaction with a more sterically demanding alcohol such as 3'-O-tBDMS-thymidine resulted in a dramatically lower diastereoselectivity. These results were

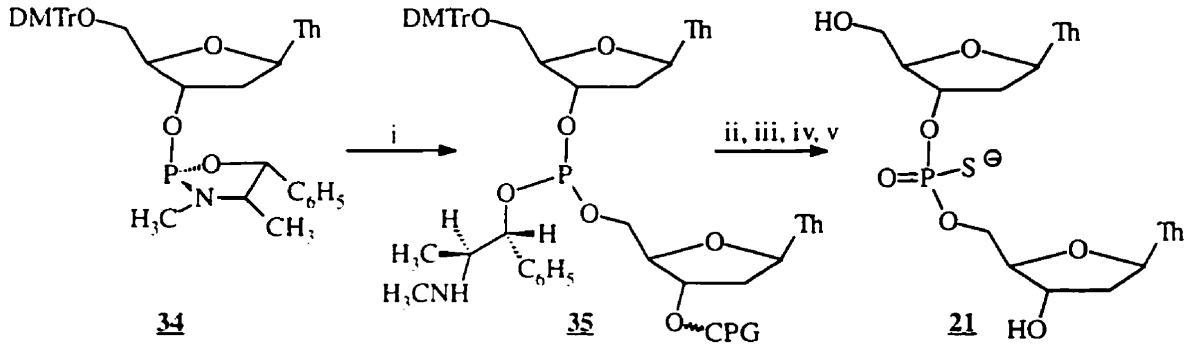
also in agreement with the mechanism proposed by Stec and Zon, since a bulkier and



Scheme 1.17: the acidic coupling of oxazaphosphorinane **31a**

more acidic catalyst was a poorer nucleophile and consequently would compete less with the incoming alcohol than tetrazole, except in the case when the incoming alcohol was more sterically demanding. This approach confirmed once again that the use of an acidic catalyst was the source of epimerization.

Iyer *et al.*[150] also described a cyclic phosphoramidite derivative **34** (scheme 1.18),



i. 3'-supported thymidine, tetrazole, CH_3CN; ii. Beaucage's reagent;
iii. Ac_2O, C_5H_5N; iv. Cl_2CHCO_2H; v. 28% NH_4OH, 55°C, 4 h

Scheme 1.18: the use of ephedrine as a precursor

synthesized from (1*R*, 2*S*)-ephedrine, and noticed that the nucleophilic opening of the oxazaphospholidine ring catalyzed by tetrazole also resulted in a very poor diastereoselectivity (ratio 2:3 of phosphite triesters **35**). Their chiral auxiliary was removed, after acetylation, under standard conditions (28% NH_4OH, 55°C) at the end of the synthesis to yield the desired phosphorothioate dimer **21**.

[150] Iyer, R. P.; Yu, D.; Ho, N. H.; T, W.; Agrawal, S. *Tetrahedron: Asymmetry* **1995**, *5*, 1051

The use of cyclic phosphoramidite derivatives as described in this section opened a new possible way of controlling the diastereoselectivity during the formation of phosphorothioate internucleotidic linkages, constituting the starting point of our studies. The plan of study and our research in that respect will be described in the following chapters.

2. Imidazo-oxazaphosphorines as precursors to chiral phosphite triesters

2.1. Plan of study

Our goal was to develop a method to synthesize diastereomerically pure P-chiral DNA analogues, particularly DNA phosphorothioates. As seen in section I.4.3., modification of phosphoramidite chemistry by Xin and Just[148,149] led to some diastereoselectivity, which was attenuated by the use of an acidic catalyst such as tetrazole.

We retained the idea of using a six-membered ring containing the N-P-O part of the phosphoramidite precursor, because of its ability to orient the stereochemistry of the phosphorus center. However, we wanted to eliminate the use of the troublesome acidic catalyst which in all cases was the source of the epimerization at the phosphorus atom.

In doing so, we needed to find another way of activating the nitrogen part of the precursor, or to make it a better leaving group. This would enable us to retain the high reactivity of trivalent phosphorus derivatives that allowed the development of solid-phase DNA synthesis[131,141].

We therefore considered the replacement of the the amine moiety of the phosphoramidite precursor by an azole, in other words incorporating the catalyst into the precursor. This should in principle eliminate the need for an acidic catalyst, since an azole would be a much better leaving group than the usual diisopropylamine.

In the literature, several examples of compounds containing an azole substituent on a trivalent phosphorus derivative have been reported, some of which are summarized in scheme 2.1. Zhang *et al.*[151] recently reported the use of 3-nitro-1,2,4-triazole as a good leaving group on **36**, requiring no acid catalysis. Grachev et al.[152] reported the synthesis of chiral dioxaphosphorinane **37** having an imidazole group as the exocyclic substituent on the phosphorus atom, obtained by substitution of a chloro group with sodium imidazolide.

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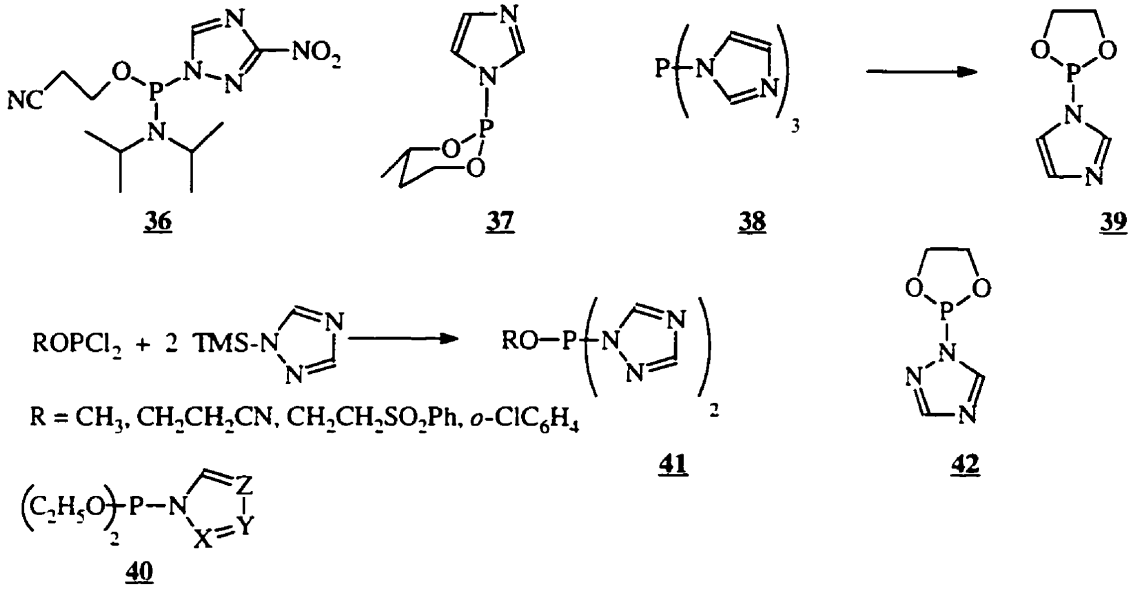
[151] Zhang, Z.; Tang, J.Y. *Tetrahedron Lett.* **1996**, *37*, 331

[152] Grachev, M. K.; Iorish, V. Y.; Bekker, A. R.; Nifant'ev, E. E. *Zh. Obshch. Khim.* **1990**, *60*, 66

Shimidzu *et al.*[153] reported the use of tri(imidazol-1-yl)phosphine **38**, a new efficient



Scheme 2.1: trivalent phosphorus derivatives bearing azole substituents

phosphorylating reagent that reacted very quickly with 1,2- or 1,3-cis diols to give cyclic dialkoxyphosphorimidazolides such as **39**. Fourrey and Varenne[154,155] reported the synthesis of bis(triazolyl) alkyl-phosphites **41**, as phosphoramidite analogues for solid phase synthesis. Kricheldorf *et al.*[156] reported a similar synthesis of dialkylphosphorotriazolide **42** useful in the synthesis of polypeptides. Finally, Berner *et al.*[145,146] reported the synthesis of a variety of diethylphosphoroazolides **40** that were used to elucidate the mechanism of phosphoramidite coupling involving a nucleophilic participation from tetrazole.

Considering the difference in pKa of pyrrole, imidazole, triazole and tetrazole, we thought their leaving group ability would be in the order tetrazole > triazole > imidazole > pyrrole. We chose to use imidazole as a leaving group, as reported several times in the literature, because of its high stability as opposed to pyrrole, which is prone to oxidation. Tetrazole was eliminated since it would be too good a leaving group. Triazole was the

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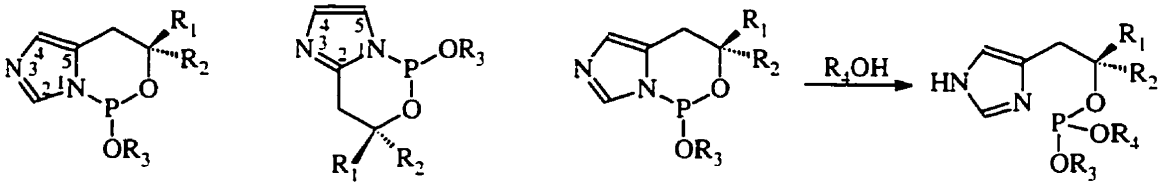
[153] Shimidzu, T.; Yamana, K.; Kanda, N.; Kitgawa, S. *Bull. Chem. Soc. Jpn* **1983**, *56*, 3483
[154] Fourrey, J. L.; Varenne, J. *Tetrahedron Lett.* **1983**, *24*, 1963
[155] Fourrey, J. L.; Varenne, J. *Tetrahedron Lett.* **1984**, *25*, 4511
[156] Kricheldorf, H. R.; Fehrle, M.; Kaschig, J. *Angew. Chem. Int. Ed. Engl.* **1976**, *15*, 305

other possible choice, but we chose imidazole since a chiral auxiliary containing the imidazole moiety could be obtained enantiomerically pure by derivatization of histidine.

Therefore, our initial goal was to study the synthesis, stability and stereo-



43 **44** **43** **45**

R_1, R_2, R_3, R_4 = H or alkyl groups

Scheme 2.2: imidazo-oxazaphosphorines

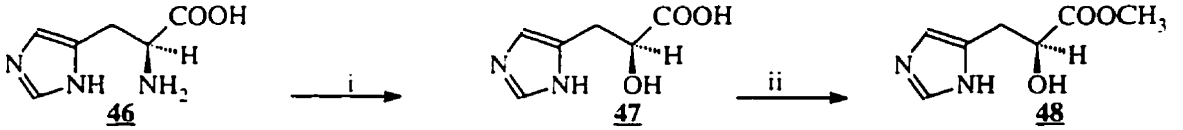
chemistry of imidazo-oxazaphosphorines of type **43** being substituted at positions 1 and 5 of the imidazole ring (scheme 2.2). We would then study the diastereoselectivity of displacement of the imidazole moiety to yield phosphite triester **45**.

As we shall see in the following section, the use of imidazo-oxazaphosphorine **44** turned out to be more convenient and the displacement of the imidazole group on this precursor was studied more thoroughly.

2.2. Synthesis and characterization of imidazo-oxazaphosphorines

2.2.1. Imidazo-oxazaphosphorine fused at positions 1 and 5 of the imidazole ring

In order to synthesize a compound like **43** (scheme 2.2) substituted on positions 1 and 5 of the imidazole ring, the obvious starting compound was histidine, since the stereochemistry was already built in the molecule and the imidazole substituted at the desired positions. Following a procedure reported by Noordam *et al.*[157] (scheme 2.3), (-)-



46 **47** **48**

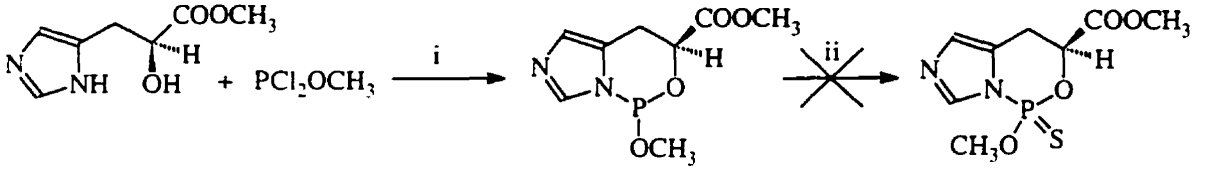
i. 1.5 $NaNO_2$, 1.5 HCl, H_2O; ii. HCl(g), CH_3OH, 0°C

Scheme 2.3: synthesis of an imidazolylpropanol derived from histidine

[157] Noordam, A.; Maat, L.; Beyerman, H. C. *Recl. Trav. Chim. Pays-Bas* **1981**, 441

L-histidine **46** was first diazotized with 1.5 eq. sodium nitrite in the presence of 1.5 eq. aqueous hydrochloric acid, in order to substitute the amino group by a hydroxyl function with retention of configuration at the stereogenic center. This stereoconservative process has been known for several decades[158] and can be applied to a large variety of amino-acids. The resulting (*S*)-2-hydroxy-3-(imidazol-4-yl)-propionic acid **47** was then esterified by methanol in the presence of dry gaseous hydrogen chloride at 0°C. The product, methyl (*S*)-2-hydroxy-3-(imidazol-4-yl)-propionate **48**, was obtained in 70% yield starting from histidine. The intermediate acid **47** was not isolated before esterification since this did not increase the yield or purity of **48**. The ester was recrystallized from a mixture of ethanol and diethyl ether, m.p. 139-142°C, $[\alpha]_{D,298}$ -21° (c 1.9, methanol) (lit. -22°).

Ester **48** was then reacted with methyl dichlorophosphite in the presence of triethylamine to neutralize hydrogen chloride formed (scheme 2.4). The reaction was



48 **49** **50**

i. 3.3 Et_3N, CH_2Cl_2, 0°C to RT; ii. S_8

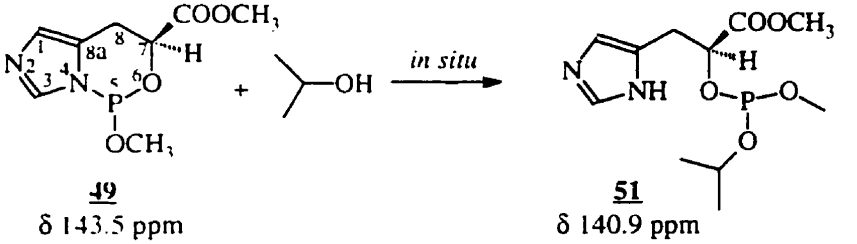
Scheme 2.4: synthesis of imidazo-oxazaphosphorine derived from histidine

carried out at 0°C in a dry NMR tube and monitored by ^{31}P NMR. The resulting imidazo-oxazaphosphorine **49** was extremely moisture sensitive, and TLC simply produced very long spots that could not be interpreted reliably. Therefore, TLC was not used as an analytical tool with these highly reactive compounds. Instead, ^{31}P NMR was generally used to monitor this type of reactions. At the beginning of the reaction, ^{31}P NMR indicated the presence of a large number of compounds having resonance signals between 176 and 120 ppm, which is an area that normally contains alkyl dichlorophosphites, dialkyl chlorophosphites, phosphite triesters, phosphoramidites, and chlorophosphoramidites. After overnight reaction at ambient temperature, the initially cloudy solution was clear and ^{31}P NMR showed a single resonance signal at 143.5 ppm. Only traces of hydrolysis

[158] Baker, C. G.; Meister, A. *J. Am. Chem. Soc.* **1951**, *73*, 1336

products such as H-phosphonates (which usually appear between 0 and 20 ppm) were observed. This compound, (*7S*)-7-carboxymethyl-7,8-dihydro-5-methoxy-imidazo[4,3-e]oxazaphosphorine, appeared as a single peak by ^{31}P NMR, corresponding to the signal of a single diastereomer or to the superimposed signals of two diastereomers. In principle, two diastereomers should have given rise to two distinct peaks, as a result of their difference in physico-chemical properties[159]. However, imidazo-oxazaphosphorine **49** could not be isolated due to its high reactivity. An attempt to sulfurize it with elemental sulfur to **50** resulted in a complex mixture of compounds having resonance peaks between 0 and 60 ppm. Bicyclic **49** could not be distilled after dilution in dry diethyl ether to precipitate triethylammonium chloride. Over time, it decomposed, giving rise to a series of compounds having resonance peaks between 0 and 15 ppm (hydrolysis or oxidation products). The same result was obtained when a drop of water was injected into the reaction mixture. Scaling up the reaction to 500 mg of precursor **48** gave essentially the same results.

We then attempted to open the oxazaphosphorine ring of **49** with an alcohol *in situ* (scheme 2.5). The synthesis of imidazo-oxazaphosphorine **49** was repeated and after



49
δ 143.5 ppm

51
δ 140.9 ppm

Scheme 2.5: opening of imidazo-oxazaphosphorine **em29**

overnight reaction, 1.2 eq. of isopropanol was injected into the NMR tube. After 15 min., ^{31}P NMR indicated the presence of a single signal at 140.9 ppm, revealing that a single phosphite triester **51** had been obtained. This seemed to indicate that the displacement of the imidazole ring had occurred in a high diastereoselective fashion. However, phosphite triester **51** decomposed when trying to isolate it by flash chromatography. Figure 2.1 summarizes these observations.

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[159] Juaristi, E. "*Introduction to Stereochemistry and Conformational Analysis*", Ed. John Wiley & Sons, New York **1991**, pp. 286-298

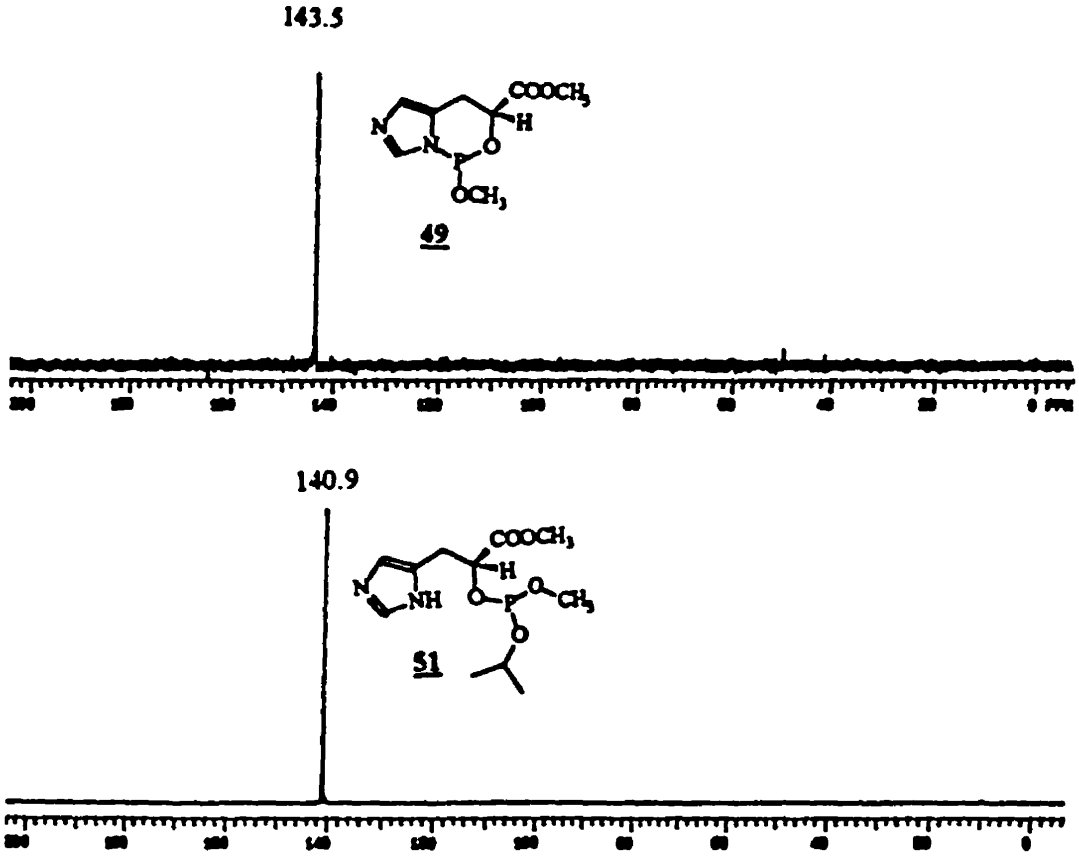


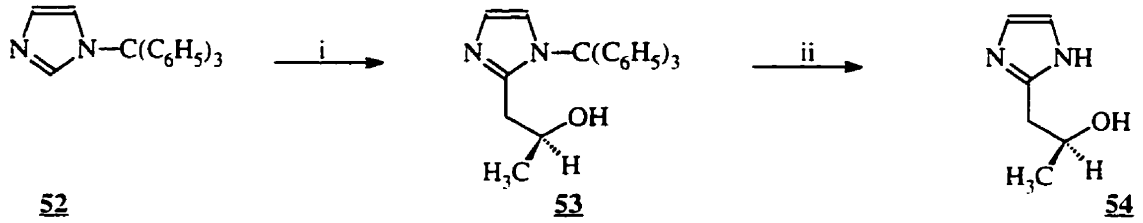
Figure 2.1: ^{31}P NMR spectra of imidazo-oxazaphosphorine **49** (top) and its transformation to phosphite triester **51** (bottom)

From this experiment, we discovered that imidazo-oxazaphosphorine **49** could be formed. However, we did not succeed in isolating it due to its reactivity. The only sign of its presence was a single ^{31}P NMR frequency at 143.5 ppm, suggesting a single diastereomer. Its opening also revealed a highly diastereoselective reaction, giving rise to the corresponding phosphite triester **51** at 140.9 ppm.

In parallel to this system we tried another similar type of imidazo-oxazaphosphorine such as **44**, lacking the ester function and in which the imidazole group was fused at positions 1 and 2.

2.2.2. Imidazo-oxazaphosphorine fused at positions 1 and 2 of the imidazole ring

In order to synthesize (*S*)-1-(imidazol-2-yl)-propan-2-ol **54** (scheme 2.6), N-tritylimidazole **52** was first deprotonated with *n*-butyllithium at -78°C, then reacted



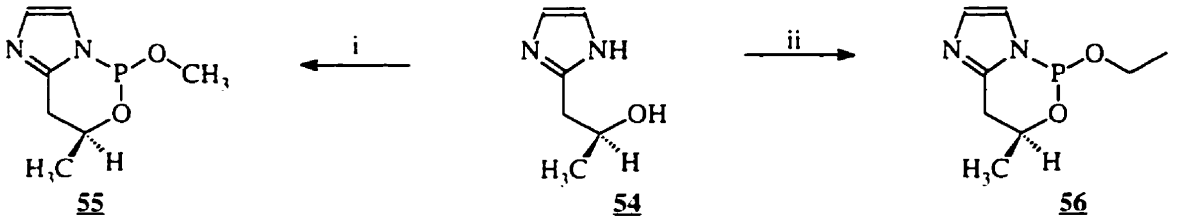
52 **53** **54**

i. BuLi, THF, -78°C then (*S*)-propylene oxide, -78°C to 0°C, 16h; ii. 5% CH_3CO_2H in CH_3OH, Rfx

Scheme 2.6: synthesis of an imidazolylpropanol derived from imidazole

with (*S*)-propylene oxide at -78°C to 0°C for 16h, as adapted from a procedure described by Kirk[160]. The product, (*S*)-1-(N-triphenylmethyl-imidazol-2-yl)-propan-2-ol **53**, was then deprotected in the presence of 5% acetic acid in refluxing methanol, and after purification by ion-exchange chromatography pure (*S*)-1-(imidazol-2-yl)propan-2-ol **54** was obtained in 75% yield starting from N-tritylimidazole.

Imidazolylpropanol **54** was then reacted with methyl dichlorophosphite or ethyl dichlorophosphite in methylene chloride at 0°C in the presence of 5.0 eq. of triethylamine (using a smaller amount of base resulted in the formation of several side products), in order to yield the corresponding imidazo-oxazaphosphorines **55** and **56** (scheme 2.7). Both reactions were initially run in an NMR tube and followed by ^{31}P NMR. In both cases, the reactions were very exothermic and resulted in the dissolution of the solid starting imidazolylpropanol (insoluble in dichloromethane), as soon as the alkyl dichlorophosphite



55 **54** **56**

i. PCl_2OCH_3, 2.2 Et_3N, CH_2Cl_2, 0°C; ii. $PCl_2OC_2H_5$, 2.2 Et_3N, CH_2Cl_2, 0°C

Scheme 2.7: synthesis of imidazo-oxazaphosphorines derived from imidazole

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

was introduced into the mixture. The ^{31}P NMR spectrum immediately revealed the presence of two resonance signals, a minor and a major one. For **55**, signals were obtained at 120.6 and 118.8 ppm, and for **56** at 120.4 and 118.3 ppm. Within 20 min., the minor signal disappeared completely in favor of the major one. Neither compound could be isolated on a 30 mg or on a 500 mg scale. However, as we shall now discuss, **56** was thoroughly analyzed by means of ^{13}C NMR. It could also be used as a reactive precursor in the diastereoselective synthesis of phosphite triesters as discussed in section 2.3.

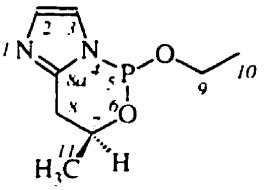
2.2.3. Conformational analysis of imidazo-oxazaphosphorine 56

The reaction leading to the formation of imidazo-oxazaphosphorine **56** was then repeated on a 50 mg scale in dry $CDCl_3$ in an NMR tube, in order to gather some conformational information on **56**. By means of ^{13}C NMR, all its resonance signals (except that of C_{8a}), as well as the signals corresponding to triethylamine, could be observed. However, ^{1}H NMR signals from **56** could not be interpreted, as they were masked by the signals of triethylamine. The APT (Attached Proton Test) experiment allowed for the unambiguous assignment of each signal. The phosphorus to carbon coupling constants could be determined as summarized in table 1 and the numbering of carbon atoms is indicated in scheme 2.8.

Table 1. ^{13}C NMR (75.3 MHz, $CDCl_3$) signals of **56**, as referred to $CDCl_3$ at 77.0 ppm

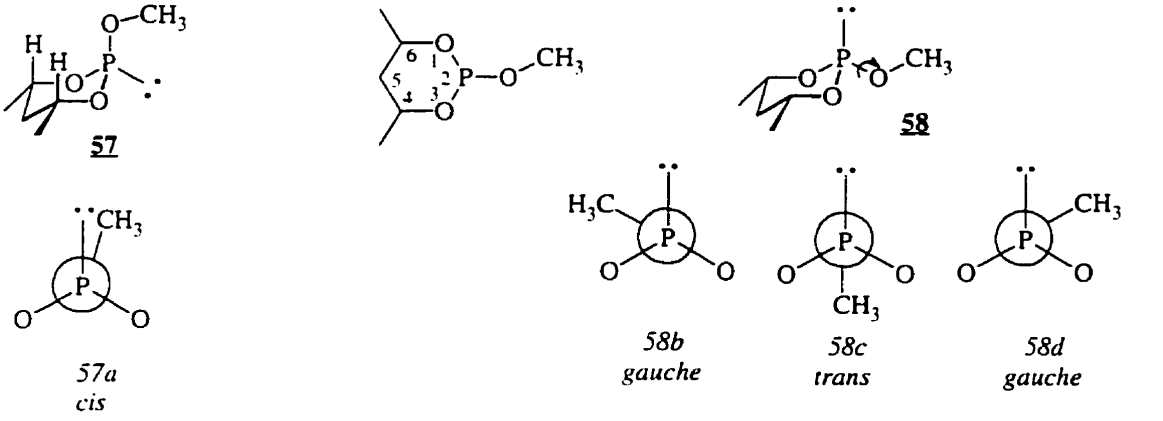
Carbon	2	3	7	8	9	10[a]	11[a]
δ (ppm)	127.57	115.07	65.94	32.74	60.23	15.53	20.38
$J_{C\text{-}P}$ (Hz)	$^{3}J = 5.2$	$^{2}J = 18.1$	$^{2}J = 6.3$	$^{3}J < 0.3$	$^{2}J = 19.4$	$^{3}J = 5.0$	$^{3}J = 4.1$

a: the assignment of C_{10} and C_{11} may be reversed



Scheme 2.8: numbering of carbon atoms on **56**

It is now well established that carbon to phosphorus coupling constants $J_{C\text{-}P}$ in trivalent phosphorus derivatives are highly dependent upon the relative spatial orientation of the lone pair on phosphorus and the carbon atom[161,162,163]. Haemers *et al.*[164,165] reported a study of the carbon to phosphorus coupling constants in a series of rigid dioxaphosphorinanes **57**-**61** (scheme 2.9). They concluded that the dihedral angle between the phosphorus lone pair and the O-C bond determines the amplitude of the $J_{C\text{-}P}$ coupling constants. A dihedral angle close to 0° will correspond to a large coupling constant, whereas a dihedral angle close to 180° will induce a very small coupling constant. For example, the coupling constant between the phosphorus atom and the



	59	57	58	60	61
$^2J_{C4\text{-}P}$	1.7 Hz	2.7 Hz	1.8 Hz	1.8 Hz	1.7 Hz
$^2J_{C6\text{-}P}$	2.2 Hz	2.7 Hz	1.8 Hz	2.1 Hz	2.2 Hz
$^2J_{CH3\text{-}O\text{-}P}$	18.3 Hz	18.2 Hz	10.7 Hz	18.7 Hz	12.0 Hz
$^3J_{C5\text{-}P}$	4.6 Hz	4.2 Hz	13.5 Hz	4.7 Hz	14.0 Hz

Scheme 2.9: conformations of dioxaphosphorinanes

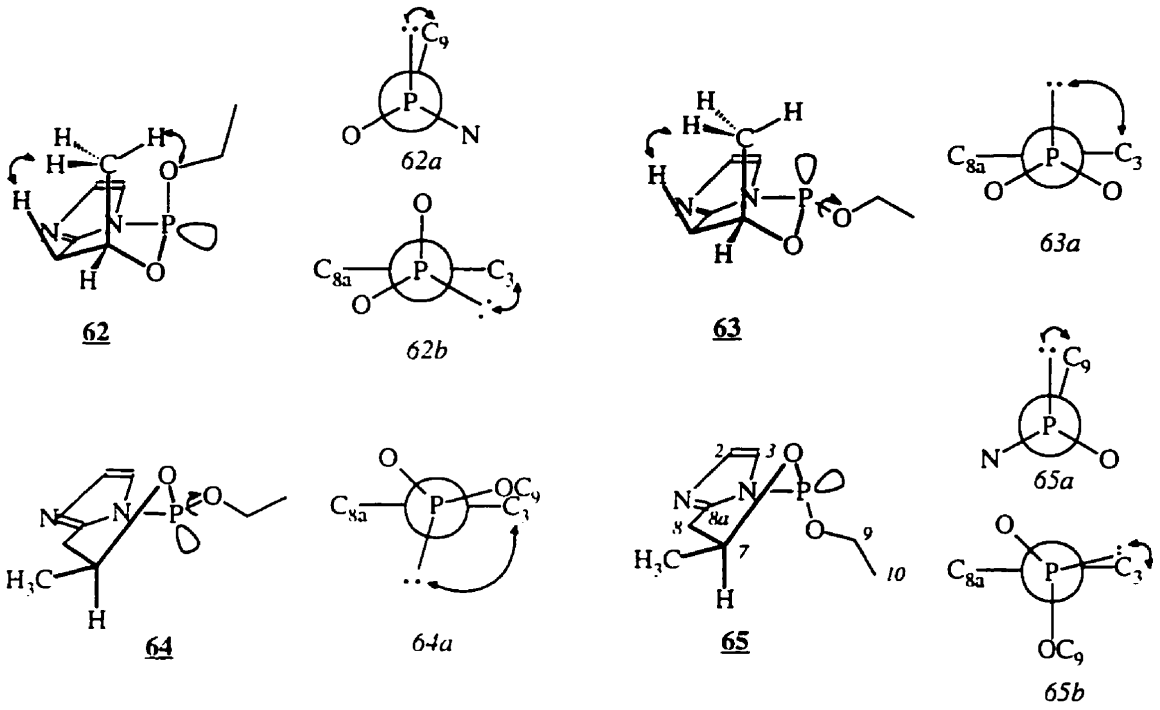
[161] White, D. W.; Bertrand, R. D.; McEwen, G. K.; Verkade, J. G. *J. Am. Chem. Soc.* **1970**, 92, 7125
[162] Breen, J. J.; Featherman, S. I.; Quin, L. D.; Stocks, R. C. *J. Chem. Soc. Chem. Commun.* **1972**, 657
[163] Sorensen, S.; Hansen, R. S.; Jakobsen, H. J. *J. Am. Chem. Soc.* **1972**, *94*, 5900
[164] Haemers, M.; Ottinger, R.; Zimmermann, D.; Reisse, J. *Tetrahedron Lett.* **1973**, *24*, 224
[165] Haemers, M.; Ottinger, R.; Zimmermann, D.; Reisse, J. *Tetrahedron* **1973**, *29*, 3538

carbon atom of the exocyclic methyl substituent on dioxaphosphorinanes **57** and **58** varies from 18.2 Hz to 10.7 Hz whether the exocyclic substituent is in the axial position as in **57** or in the equatorial position as in **58** respectively. In compound **57**, the rotation around the exocyclic P-O bond is prevented by the presence of two axial protons on the dioxaphosphorinane ring. Therefore, the most abundant rotamer will be the one having the methyl group as far as possible from the ring, eclipsed with the lone pair on the phosphorus atom (Newman projection *57a*), with a dihedral angle close to 0°. This translates in a large coupling constant ($^2J_{C\text{-}P}$ = 18.2 Hz) On the contrary, compound **58** lacks this interaction with the ring protons and the rotation around the exocyclic P-O bond is easier, resulting in a larger number of possible rotamers, as indicated by Newman projections *58b*, *58c*, *58d*. Consequently, the averaged dihedral angle between the carbon atom on the exocyclic methyl group and the phosphorus atom lone pair in compound **58** will be larger, resulting in a significantly smaller coupling constant ($^2J_{C\text{-}P}$ = 10.7 Hz). This behavior is similar in compounds **59**, **60** and **61**.

If we now consider carbon atoms 4 and 6 of each compound, the dihedral angle between the O-C_4 or O-C_6 bond and the phosphorus lone pair is small, whether the exocyclic substituent is axial or equatorial. In the former case, the dihedral angle between the phosphorus atom lone pair and the O-C_4 or O-C_6 bond is close to 180° (*trans* orientation). In the latter, the same dihedral angle is near 60° (*gauche* orientation). The $^2J_{C4\text{-}P}$ and $^2J_{C6\text{-}P}$ coupling constants remained low in all these compounds. Consequently, the authors concluded that the $J_{C\text{-}P}$ coupling constant presents a very steep decrease when the angle varies from 0 to 60°, then remains low from 60 to 180°. Finally, the $^3J_{C5\text{-}P}$ coupling constant is larger in the compounds bearing an equatorial substituent, as opposed to these having an axial substituent.

In structure **56**, the six-membered ring cannot exist in a true chair conformation, due to the insaturation at C_{8a}. The ring is flattened at that position, forcing C_8 to be in the same plane as the imidazole ring. Additionally, the anomeric effect will contribute to orient the exocyclic ethoxy substituent at phosphorus in the pseudo-axial position, as already established by several authors on the study of conformations of dioxaphosphorinanes and oxazaphosphorinanes. Considering the observed coupling constants $^2J_{C9\text{-}P}$ = 19.4 Hz and

$^2J_{C3\text{-}P}$ = 18.1 Hz, both dihedral angles between C_9 and the phosphorus lone pair and between C_3 and that same lone pair should be small in order to reflect their respective large coupling constants. This rules out the possibility of a rapid inversion at the phosphorus center, which would have resulted in an averaged larger dihedral angle and consequently an averaged smaller coupling constant for both atoms. Besides, the observation of no coupling constant between C_8 and P seems to indicate that the exocyclic substituent is in an axial orientation. Scheme 2.10. summarizes the four possible conformers of **56**. Conformer **62** has the expected dihedral angle between the phosphorus atom lone pair and C_9 (*62a*)



Scheme 2.10: possible conformations of imidazo-oxazaphosphorine **56**

and C_3 (*62b*). However, it is sterically highly strained, due to the presence of the methyl group in pseudo-axial position, which shows an eclipsing interaction with the proton on C_8 and a strong 1,3-diaxial interaction with the oxygen atom of the ethoxy group. Conformer **63** is less sterically compressed than **62**, since the 1,3-diaxial interaction with the ethoxy substituent has disappeared. However, it should display a small $^2J_{P\text{-}O\text{-}C7}$ coupling constant due to the presence of several rotamers around the exocyclic P-O bond. Moreover, the dihedral angle between the phosphorus atom lone pair and C_3 (*63a*) is not minimal.

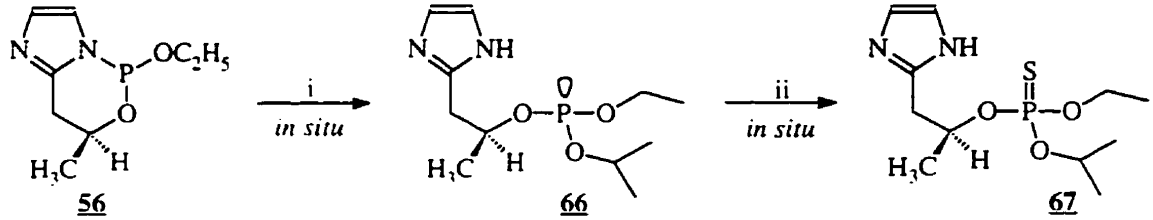
Conformer **64** does not display the unfavorable steric features of **62** and **63**. Here again, the rotation around the exocyclic P-O bond should result in a relatively large average P-O-C_9 dihedral angle, and thus in a smaller coupling constant. Likewise, the P-N-C_3 (*64a*) dihedral angle is not minimal. Finally, conformer **65** seems to have all the characteristics corresponding to the most stable structure. It is not highly strained like **62** and **63**, and the P-O-C_9 (*65a*) and P-N-C_3 (*65b*) dihedral angles are both very small, which experimentally translates into a large coupling constant. Therefore, **65** is the conformation proposed for (*7S*)-7,8-dihydro-5-ethoxy-7-methyl-imidazo[3,4-a]oxazaphosphorine **56**.

2.3. Imidazo-oxazaphosphorines as precursors to chiral phosphite triesters

2.3.1. Displacement of the imidazole group

As described in sections 2.2.1 and 2.2.2, imidazo-oxazaphosphorines substituted in positions 1,5 and 1,2 of the imidazole ring turned out to be so labile that they could not be isolated. Their use as phosphitylating reagents that could be generated *in situ* before the condensation reaction with a nucleoside was then considered.

Therefore, an experiment was performed in which imidazo-oxazaphosphorine **56** was first synthesized and equilibrated to a single diastereomer as shown by ^{31}P NMR. When NMR indicated the presence of a single diastereomer, an alcohol was then injected (scheme 2.11). The first trial was carried out with isopropanol, and the result was a gradual disappearance of the starting materials having a resonance signal at 118.3 ppm. A signal then appeared at 140.6, that was in the area of saturated phosphite



56 δ 118.3 ppm

66 δ 140.6 ppm

67 δ 64.8 ppm

i. iPrOH; ii. S_8

Scheme 2.11: the diastereoselective ring opening by an alcohol

triesters (around 140 ppm). The transformation required about 20 min. to go to completion. The most important fact was that the resulting phosphite triester **66** appeared by NMR as a single resonance signal (figure 2.2), indicating its presence as a single diaste-

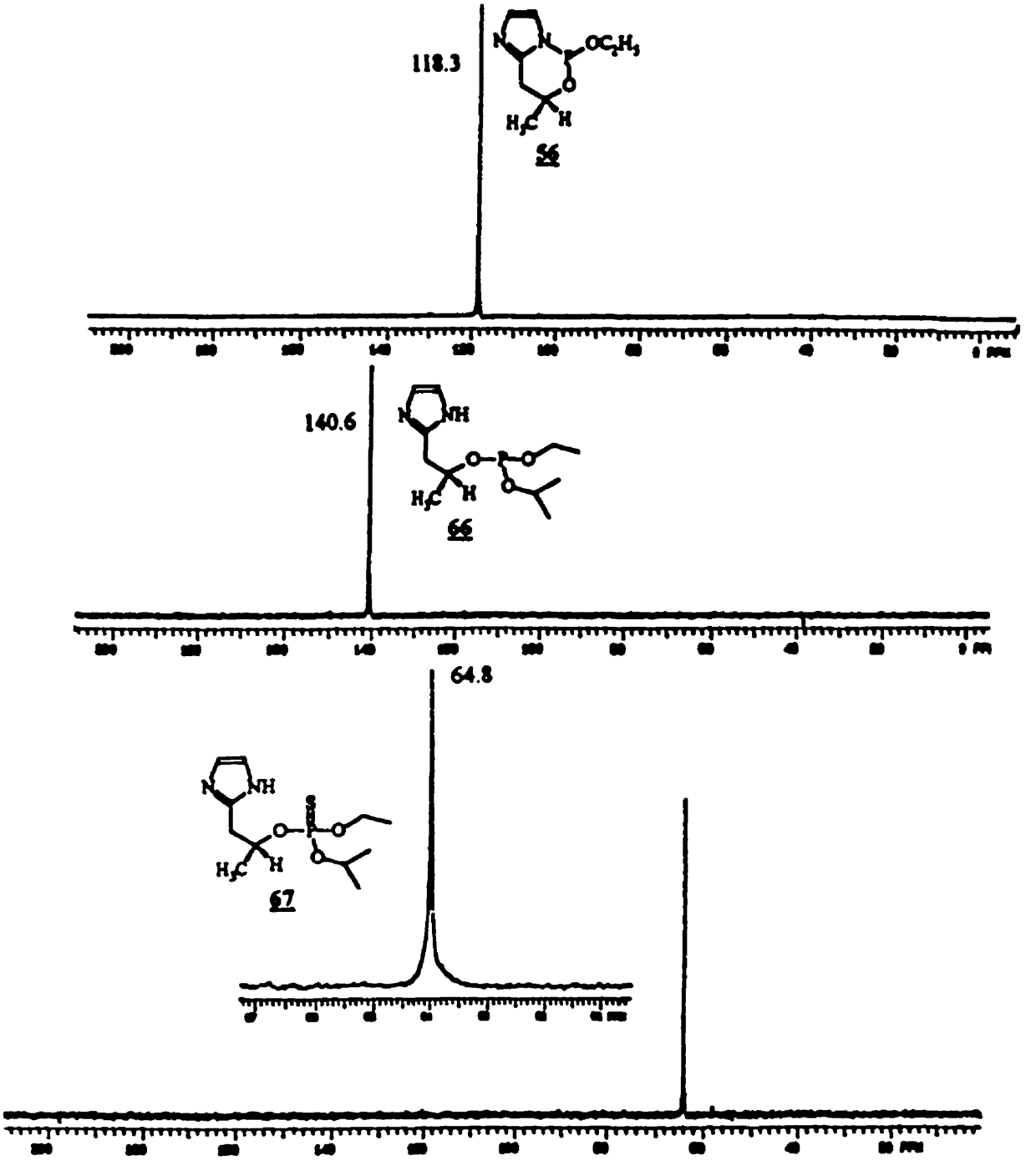
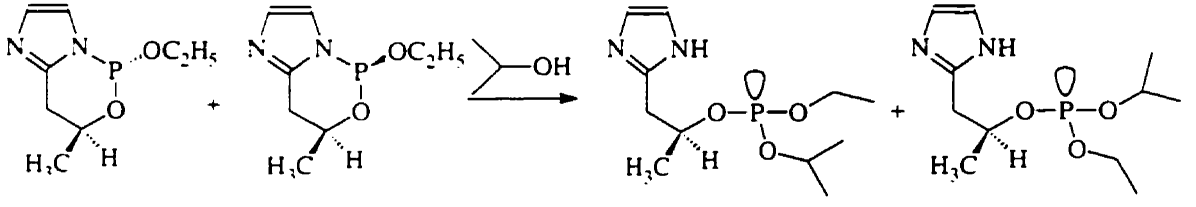


Figure 2.2: ^{31}P NMR spectra of imidazo-oxazaphosphorine **56** (top), phosphite triester **66** (center) and phosphorothioate triester **67** (bottom)

reomer, suggesting a highly diastereoselective reaction. Given the experimental error, the chiral purity of the product was evaluated to be > 98:2 by ^{31}P NMR.

Without further purification, elemental sulfur was introduced into the reaction mixture in order to convert phosphite triester **66** into the corresponding phosphorothioate triester **67**, a process known to occur with retention of stereochemistry at the phosphorus atom[166]. The signal corresponding to phosphite triester **66** disappeared within 2 min. and another single resonance peak appeared at 64.8 ppm, indicating there again the presence of a single diastereomer as expected. Phosphorothioate triester **67** was then purified and characterized.

In order to make sure that the NMR signals obtained actually corresponded to single compounds and were not the result of an overlap of the signals corresponding to two diastereomers, the previously described experiment was repeated differently. Instead of introducing the alcohol after the equilibration to a single diastereomer had occurred, it was introduced as the signals corresponding to both diastereomers of **56** were still present, within 5 min. after the introduction of ethyl dichlorophosphite into the reaction mixture. The result was, as expected (scheme 2.12), the appearance of two resonance signals for the corresponding phosphite triester **66**, the major one at 140.6 ppm as previously indicated and the minor one at 141.1 ppm, corresponding to the other diastereomer having



Scheme 2.12: displacement on a mixture of diastereomers

the opposite configuration at the phosphorus atom. This experiment confirmed that the signal observed at 140.6 ppm during the formation of **66** actually corresponded to a single diastereomer and was not the result of the superimposition of two signals, as summarized in figure 2.3.

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[166] Horner, L. *Pure Appl. Chem.* **1964**, 225

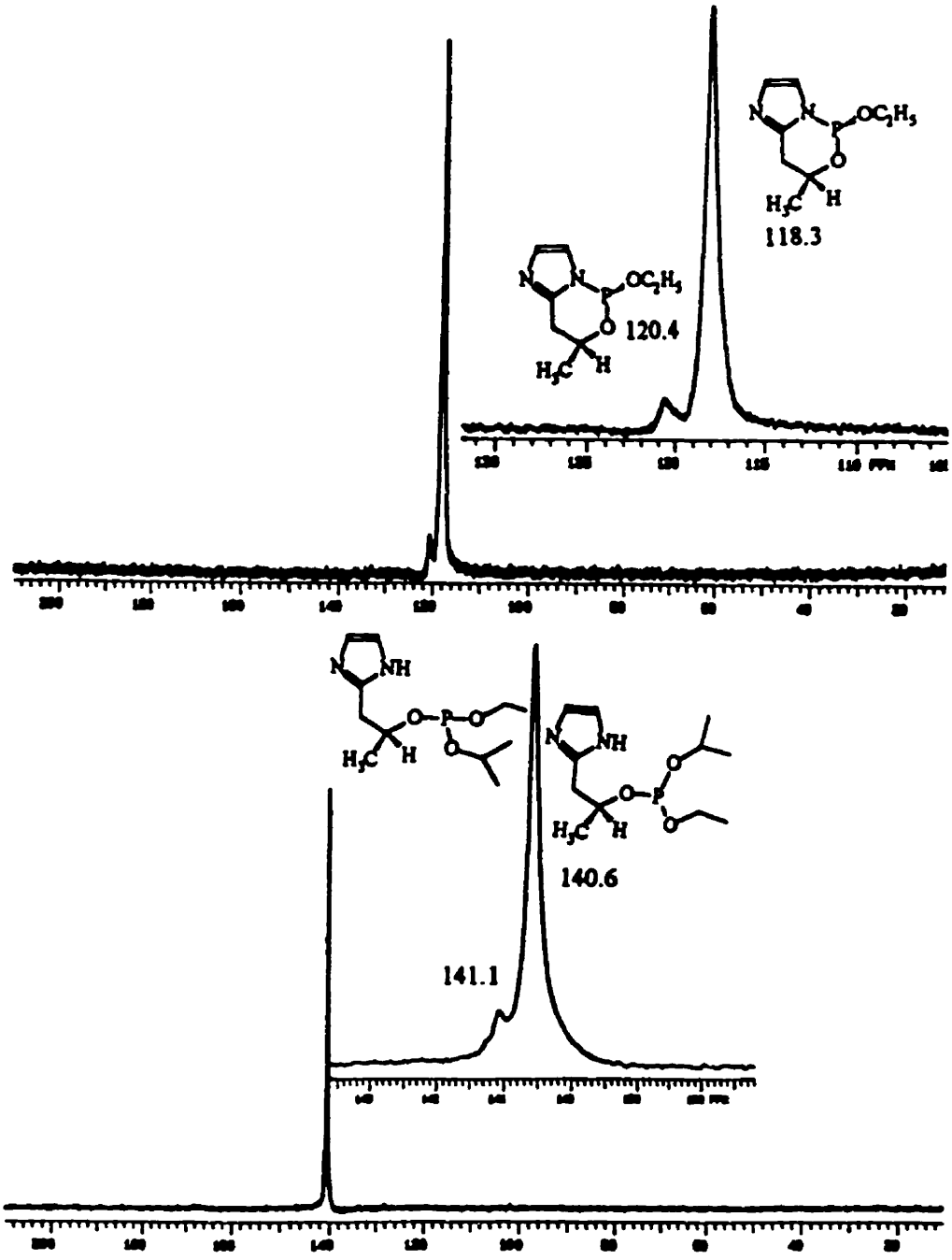


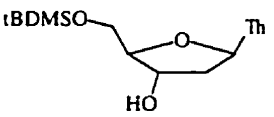
Figure 2.3: ^{31}P NMR spectra of both diastereomers of imidazo-oxazaphosphorine **56** (top), and both diastereomers of phosphite triester **66** (bottom)

2.3.2. Further application of the displacement

Several alcohols were then used to displace the imidazole ring of imidazo-oxazaphosphorine **56**. In each case, the reaction was highly diastereoselective and the speed of displacement varied as a function of the size of the nucleophile. Table 2

summarizes the chemical shifts of the phosphite triesters and phosphorothioate triesters for the various alcohols used.

Table 2: ^{31}P NMR parameters for phosphite triesters and phosphorothioate triesters (in ppm downfield from 85% H_3PO_4)

Nucleophile	Phosphorothioate triester δ (ppm)	Phosphite triester δ (ppm)	Reaction time (min.)
BnOH	**68**, 66.3	139.8	< 2
iPrOH	**67**, 64.8	140.6	20
	**69**, 66.3	141.2	30

All the phosphite and phosphorothioate triesters chemical shifts appeared in the usual range. As expected, benzyl alcohol reacted faster (< 2 min.) than a simple secondary alcohol (20 min. for isopropanol), which in turn reacted faster than a hindered cyclic secondary alcohol (30 min. for 5'-O-tBDMS-thymidine).

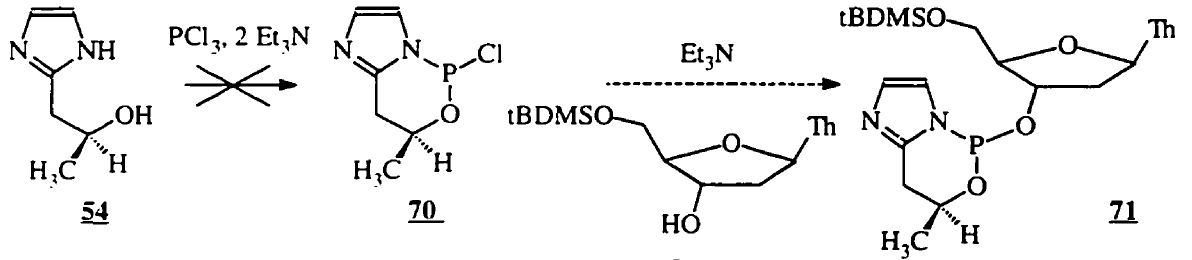
To summarize, imidazo-oxazaphosphorine **56** turned out to be a good precursor for the diastereoselective synthesis of some phosphite triesters. The synthesis of the imidazo-oxazaphosphorine moiety was relatively simple, even though the product was very reactive. The displacement of the imidazole moiety on this intermediate was highly diastereoselective as demonstrated above, and allowed for the introduction of a nucleoside group on the phosphorus atom, by reaction with 5'-protected thymidine.

In order to synthesize phosphorothioate dimers, the ethyl group in precursor **56** had to be replaced by a nucleoside, and the chiral auxiliary had to be removable at the end of the reaction sequence. The following section describes our efforts to address the first requirement.

2.4. Introduction of a nucleoside on the exocyclic position

2.4.1. Direct approach

Having shown that imidazo-oxazaphosphorine **56** could be formed as a single diastereomer and its reaction with an alcohol could yield simple phosphite triesters in a highly diastereoselective fashion (sections 2.2 and 2.3), the principle on which our approach would be based was established. The next step was to introduce a nucleoside moiety on the exocyclic position of the oxazaphosphorine ring, in order to form imidazo-oxazaphosphorine **71** (scheme 2.13). The plan was then to react this bicyclic precursor with 3'-O-tBDMS-thymidine as presented in section 2.3. The synthesis of **71** was attempted in the usual way[167,168,169] leading to P-substituted dioxaphosphorinanes and oxazaphosphorinanes. We envisaged to carry out the reaction of imidazolylpropanol **54** on



i. PCl_3, 2.2 Et_3N, CH_2Cl_2; ii. 5'-O-tBDMS-thymidine, 1.1 Et_3N

Scheme 2.13: direct approach to the introduction of an exocyclic nucleoside

phosphorus trichloride in the presence of 2 eq. of triethylamine to form imidazo-oxazaphosphorine **70** having a chloro substituent on the phosphorus atom. This chloro group was then supposed to be displaced by reaction of **70** with 5'-O-tBDMS-thymidine in the presence of 1 eq. of triethylamine to yield the desired **71**.

However, upon reaction of imidazolylpropanol **54** with phosphorus trichloride in the presence of 2 to 10 eq. of triethylamine, a complex mixture of compounds was obtained, having resonance signals between 219 and 140 ppm as observed by ^{31}P NMR. The ^{31}P NMR spectrum of this mixture indicated no change after several hours, as well as

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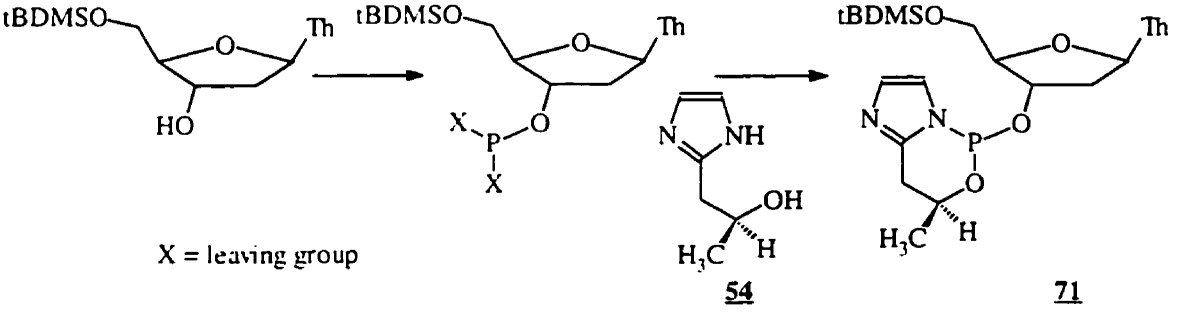
[167] Bentrude, W. G.; Tan, H. W.; Yee, K. C. *J. Am. Chem. Soc.* **1975**, *97*, 573
[168] Huang, Y.; Yu, J.; Bentrude, W. G. *J. Org. Chem.* **1995**, *60*, 4767;
[169] Gordillo, B.; Garduño, C.; Guadarrama, G.; Hernández *J. Org. Chem.* **1995**, *60*, 5180

upon heating to 40°C for a few hours. On several occasions, the mixture turned into a single compound having a resonance signal at 225 ppm by ^{31}P NMR after 48h at RT. This compound could not be isolated, but its resonance frequency did not correspond to the desired **70**. Besides, this unknown gave many products upon reaction with an alcohol and its formation was not very reproducible. Various bases were then used to perform this reaction (triethylamine, diisopropylethylamine, pyridine), as well as different solvents (dichloromethane, chloroform, diethyl ether and pyridine) and temperatures (-78°C, 0°C, RT, 50°C). Each time the reaction was followed by ^{31}P NMR and results were similar to the ones mentioned above, or hydrolysis was observed, giving compounds having resonance signals around 0 to 20 ppm.

Consequently, this approach could not be used to synthesize the desired **71** possessing a thymidine residue on the phosphorus atom.

2.4.2. Synthesis via 3'-activated nucleoside

Alternate ways were then considered for the synthesis of imidazo-oxazaphosphorine **71**. The starting 5'-protected nucleoside would first be functionalized at the 3'-position in order to yield a "3'-activated" nucleoside derivative bearing two leaving groups at the phosphorus atom. This precursor would then react with imidazolylpropanol **54** to yield the desired imidazo-oxazaphosphorine **71**. Scheme 2.14 summarizes this

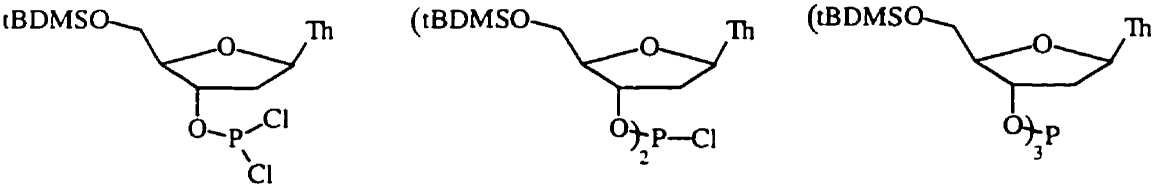


Scheme 2.14: introduction of a nucleoside via 3'-functionalization

approach. Various functionalities could be considered as leaving groups for this strategy. The choice of a good leaving group was influenced by the high sensitivity of imidazo-oxazaphosphorine **71** to moisture and acidic conditions (its analogue **56** was much more

sensitive to these conditions than phosphoramidites, as demonstrated in section 2.3). Therefore, the X leaving group had to leave under mild, non acidic conditions.

The direct reaction of 5'-O-tBDMS-thymidine or 5'-O-DMTr-thymidine with phosphorus trichloride in ether or dichloromethane, at -78°C, 0°C or RT in the presence of a base and at different concentrations gave a mixture of products of mono-, di- and trisubstitution **72**, **73**, **74**, characterized by resonance signals at 177.0, 165.1 and 151.4 ppm (scheme 2.15). The major product was the monosubstitution product, which constituted only 60 to 70% of the mixture, as evaluated from ^{31}P NMR. This mixture did not display any change to a single compound after standing at RT or mild heating for



72
δ 177.0 ppm
60-70%

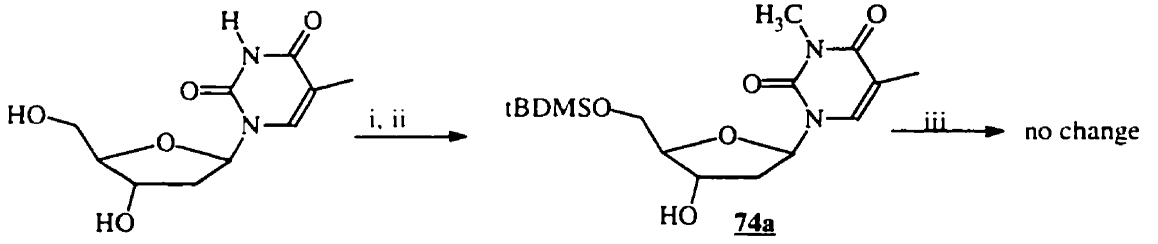
73
δ 165.1 ppm

74
δ 151.4 ppm

Scheme 2.15: direct reaction with phosphorus trichloride

several hours, and decomposed upon prolonged standing at ambient or low temperature. Figure 2.4 summarizes these results.

In order to make sure the acidic NH residue from the thymine base did not interfere with the reaction, this position was selectively methylated in the presence of trimethyl phosphate in a buffered solution (scheme 2.16), then silylated at the 5'-position to yield **74a**. The direct reaction with phosphorus trichloride was attempted again, and gave essentially the same results. Therefore, the chloro group, often used as a good



i. $(CH_3O)_3P=O$, H_2O, NaOH; ii. tBDMSCl, imidazole, DMF; iii. PCl_3, Et_3N, CH_2Cl_2

Scheme 2.16: the use of 3-methyl thymidine **74a**

leaving group, was eliminated at the early stage of this part of the work. The following sections will present the results obtained when dimethylamine, imidazole and substituted phenoxides were used as leaving groups.

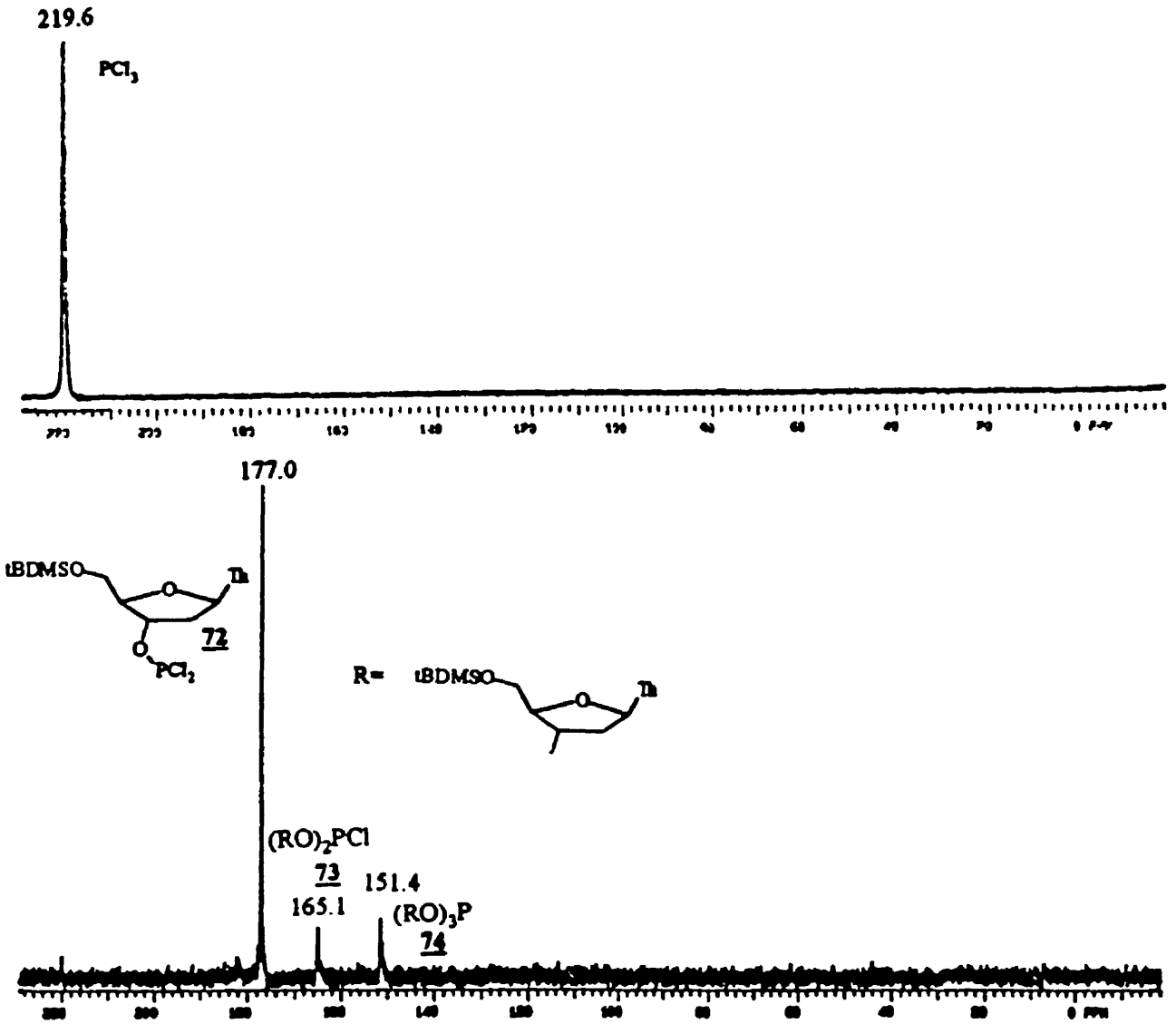
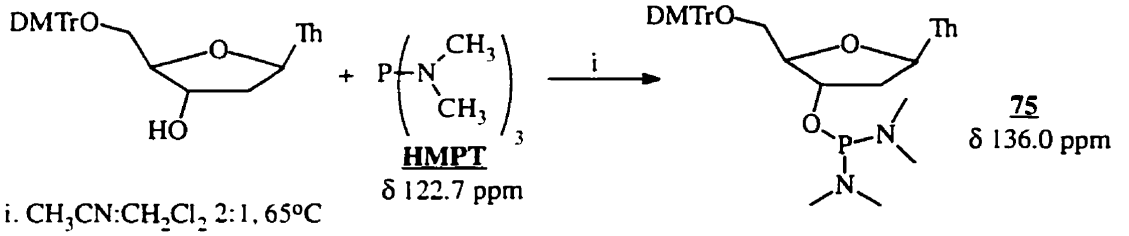


Figure 2.4: ^{31}P NMR spectra of PCl_3 (top) and the products derived from its direct reaction with 5'-O-tBDMS-thymidine (bottom)

2.4.2.1. Dimethylamine as a leaving group

Hexamethylphosphorous triamide (HMPT) was chosen as a starting reagent, in order to functionalize 5'-O-DMTr-thymidine at the 3'-position to give phosphorodiamidite 75. The protected thymidine was introduced slowly to a solution of HMPT in dry THF, in

order to consume it before it would have time to react with phosphorodiamidite **75** produced in the reaction. At room temperature, the reaction went no further than 30%. After refluxing for 1h, ^{31}P NMR indicated that it had gone to 50% completion, as indicated by the decrease in the intensity of the peak corresponding to HMPT at 122.7 ppm and the appearance of a single peak at 136.0 ppm. Purging continuously the reaction with dry argon helped the reaction to go up to 75% completion, by expelling the dimethylamine formed, but some side-products having resonance frequencies between 15 and 25 ppm were observed. As indicated in scheme 2.17, the solvent mixture was then replaced by acetonitrile:dichloromethane 2:1 (dichloromethane helped solubilize 5'-O-DMTr-

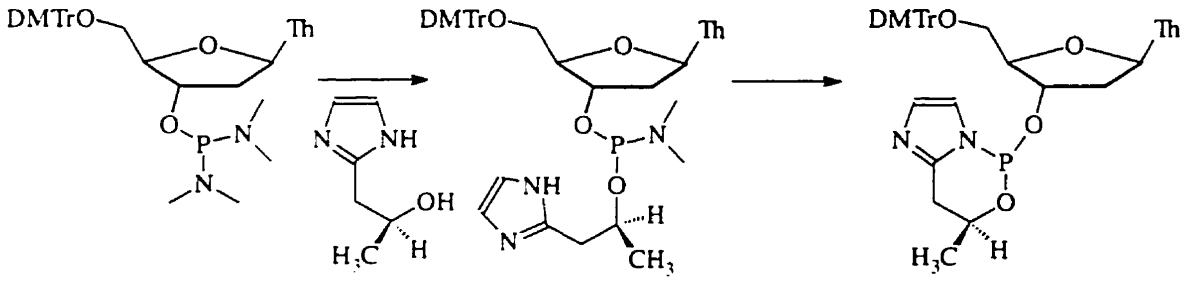


Scheme 2.17: 3'-functionalization of 5'-O-DMTr-thymidine using HMPT

thymidine), which allowed the reaction to go to completion. The yield was optimal when a solution of 5'-O-DMTr-thymidine in dichloromethane was syringed into a solution of HMPT in acetonitrile at 70°C over 1h, the mixture being purged continuously with dry argon to expel the dimethylamine formed. Reaction was complete within 30 min., giving a reasonably pure sample of **75**. However, upon chromatography most of the sample decomposed, even when the eluent contained as much as 20% triethylamine. Only 20% product was obtained, the purity of which did not exceed that of the crude mixture.

As expected, HMPT also reacted with 5'-O-DMTr-thymidine in the presence of 2 eq. of tetrazole as a catalyst (1 eq. to protonate the amine moiety and another to trap it, forming dimethylammonium tetrazolide). For this reaction, acetonitrile was used as a solvent at ambient temperature to give phosphorodiamidite **75**, which was as pure as with the previously mentioned method. However, chromatography or washing was required to separate the desired product from the salt, which resulted to a large extent in decomposition of the product.

Consequently, the next step was performed on a crude sample of phosphorodiamidite **75**, obtained by direct reaction of HMPT with 5'-O-DMTr-thymidine. The crude **75** was reacted with imidazolylpropanol **54**. A solution of imidazolylpropanol **54** in acetonitrile:pyridine 9:1 was directly injected into the reaction mixture at 70°C over 1h, pyridine being used to solubilize imidazolylpropanol **54**. There again, argon was bubbled through the solution, in order to expel the dimethylamine produced. ^{31}P NMR indicated an incomplete reaction at the end of the addition. After 1h, 4 products were observed: two at 140.6 and 140.0 ppm in a ratio of 3:1, constituting about 70% of the mixture, and two at 120.6 and 120.1 in a ratio of 1:12, constituting about 30% of the mixture. The first two were attributed to the two diastereomers of compound **76**, resulting from the displacement of one dimethylamino group on the starting materials and as shown in scheme 2.18. The second set of peaks was attributed to the desired imidazo-oxazaphosphorine **77** as a mixture of diastereomers, given that their chemical shifts were



75 δ 136.0 ppm

54

76 δ 140.6 ppm (minor) δ 140.0 ppm (major) ratio 1:3

77 δ 120.1 ppm (major) δ 120.6 ppm (minor) ratio 12:1

Scheme 2.18: reaction with imidazolylpropanol

very similar to that of their analogue **56** bearing an ethyl group on the phosphorus atom (δ 118.3 ppm, scheme 2.7). The starting phosphorodiamidite **75** was completely consumed at that time, as shown by the disappearance of its NMR signal at 136.0 ppm. The presence of two compounds around 140 ppm ruled out the possibility of having formed a phosphite triester bearing two identical alkoxy substituents, since this product should exist only as one diastereomer and therefore display a single NMR signal. Refluxing the mixture longer did not lead to any significant increase in the amount of the desired product, nor to any

change in the relative ratio of both diastereomers of 77. It simply led to a larger amount of hydrolysis products having resonance signals between 0 and 25 ppm, as summarized in figure 2.5.

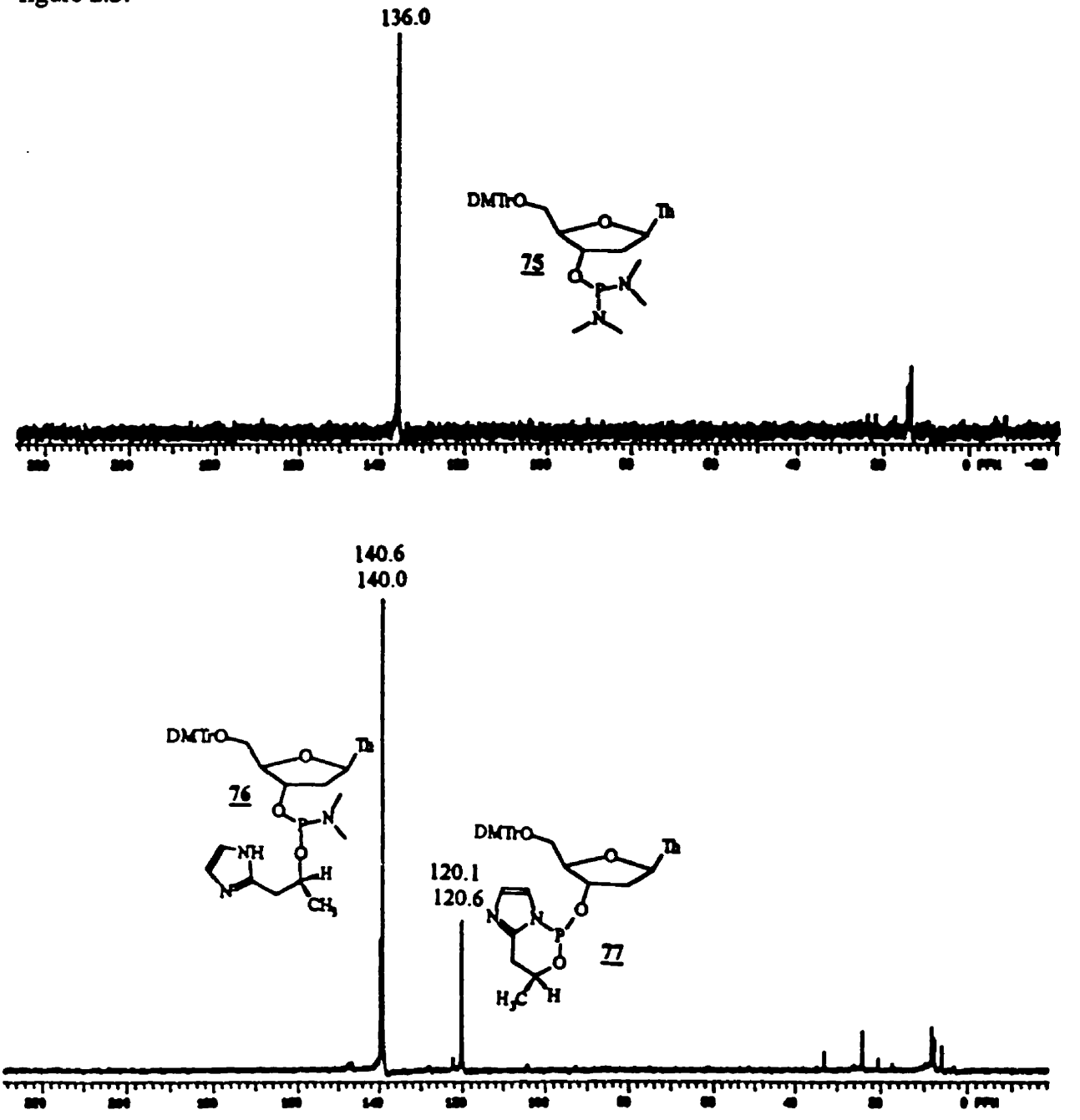
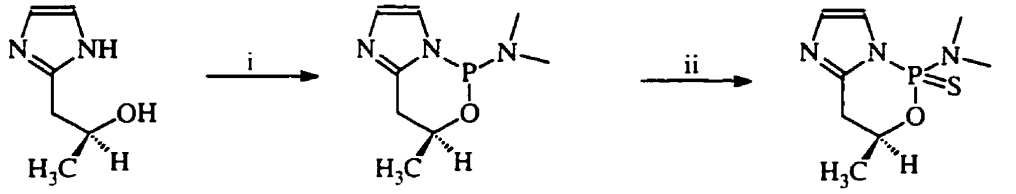


Figure 2.5: ^{31}P NMR spectra of phosphorodiamidite 75 (top) and its reaction products with imidazolylpropanol 54 (bottom)

Therefore it seemed that the amine moiety was difficult to displace by an imidazole group, even though dimethylamine was expelled from the solution, preventing it from displacing the imidazole again. In order to verify this fact, the direct reaction of imidazolylpropanol **54** with HMPT was performed. HMPT was introduced into a solution of **54** in acetonitrile:pyridine 5:1 at 65°C. ^{31}P NMR indicated mostly starting materials after 30 min. It required 20h to observe the complete disappearance of the signal



54

78
δ 127.4 ppm
δ 103.8 ppm
ratio 1:2

79
δ 81.8 ppm
δ 62.4 ppm
ratio 2:1

i. HMPT, CH_3CN:C_5H_5N, Rfx; ii. S_8

Scheme 2.19: synthesis of dimethylamine-functionalized imidazo-oxazaphosphorines

corresponding to HMPT and the appearance of two compounds having signals at 127.4 and 103.8 ppm respectively, in a ratio of 1:2, corresponding to imidazo-oxazaphosphorine **78** (scheme 2.19). Various hydrolysis products accounted for 30% of the mixture, as evaluated by ^{31}P NMR.. This mixture was sulfurized in the presence of elemental sulfur, resulting in the corresponding thio derivatives **79** as two diastereomers appearing at 81.8 and 62.4 ppm in a ratio of 2:1. These diastereomers decomposed during the purification process. However, their ^{31}P NMR signals related very closely to those of both diastereomers of the very similar **100**, which could be isolated and characterized (scheme 2.30). Figure 2.6 summarizes these results.

Surprisingly, the two diastereomers of imidazo-oxazaphosphorines **78** and their thio analogues **79** had resonance signals about 20 ppm apart. Typically, pairs of diastereomers show resonance frequencies no more than 3 ppm apart from each other. The obtention of two products with an exocyclic amino group on the phosphorus atom is in agreement with studies carried out by several groups on the stereochemistry of dioxaphosphorinanes and oxazaphosphorinanes bearing various groups on the exocyclic

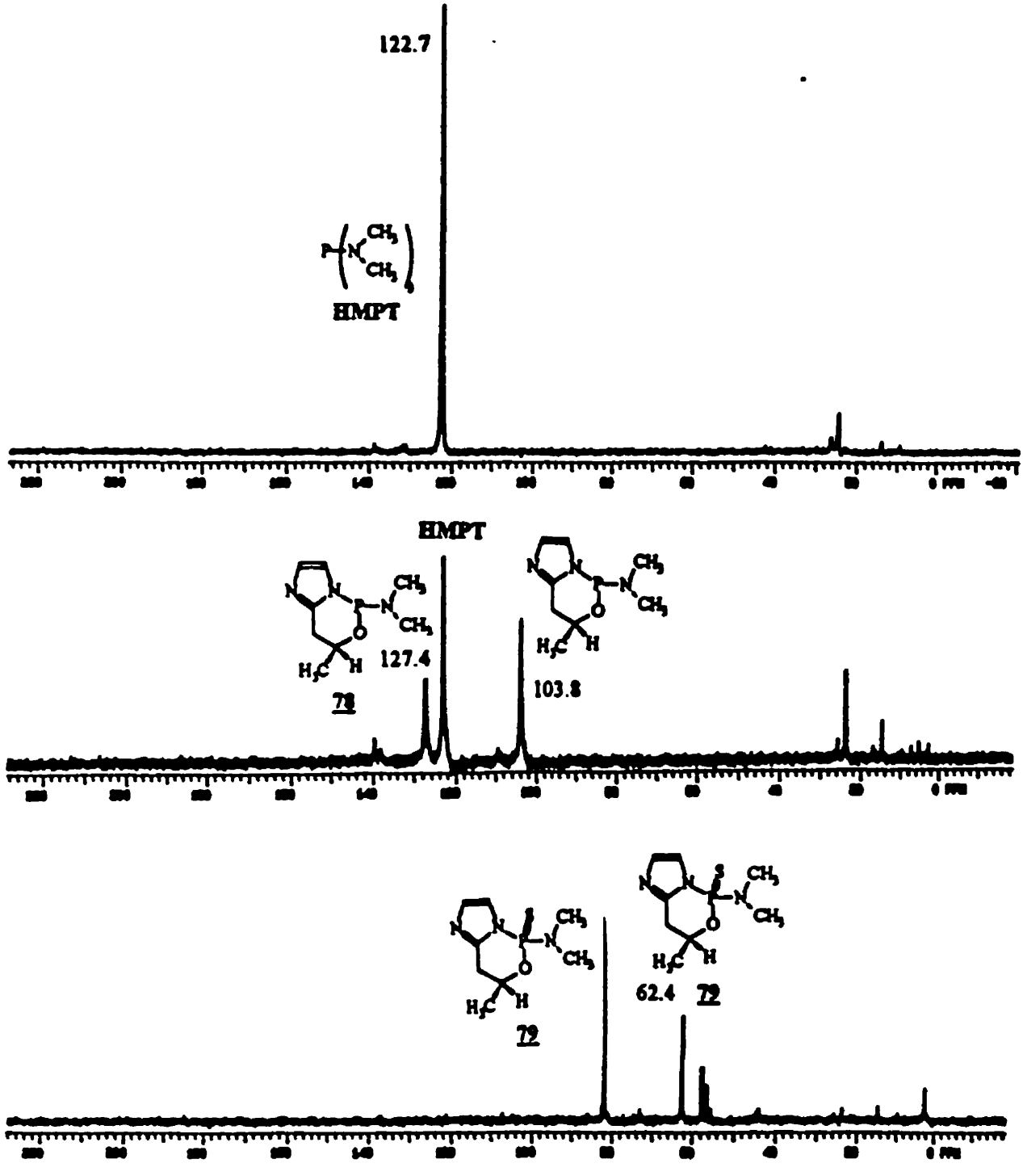
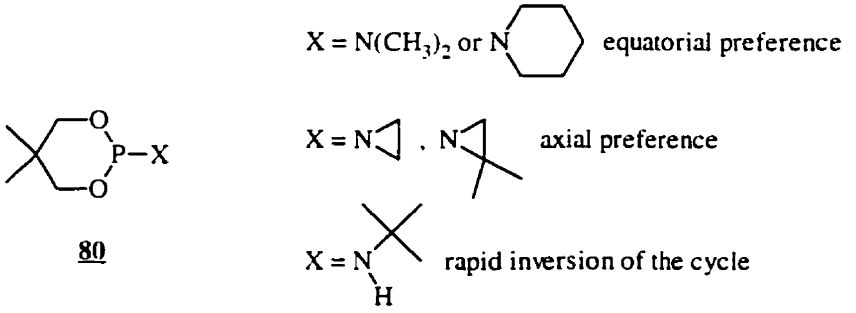


Figure 2.6: ^{31}P NMR spectra of HMPT (top), the reaction mixture after 6h during the formation of imidazo-oxazaphosphorine 78 (center), and the sulfurized product 79 after complete reaction (bottom)

position at the phosphorus atom[170,171]. When the exocyclic substituent at phosphorus was an amino group, the preferred orientation of this group was no longer axial, as observed for the more electronegative chloro or alkoxy substituent, but equatorial in certain cases, axial in some others. Scheme 2.20 summarizes the results obtained by Cogne *et al.*[4] on the study of various dioxaphosphorinanes such as **80**.



80

Scheme 2.20: amine-functionalized dioxaphosphorinanes

The fact that the displacement of the dimethylamine moiety took a long time in this case was attributed to the poor nucleophilicity of the imidazole group. Once the reaction was complete, a significant amount of hydrolysis was observed. These observations were consistent with the difficulties observed during the synthesis of imidazo-oxazaphosphorine **77**.

The main advantage of using HMPT as a starting reagent was that the side product dimethylamine could be eliminated due to its low boiling point (b.p.^{760} 7°C), leading to a compound that would not require any further purification, had the reaction been selective. Other amines did not present this advantage, therefore other leaving groups were investigated.

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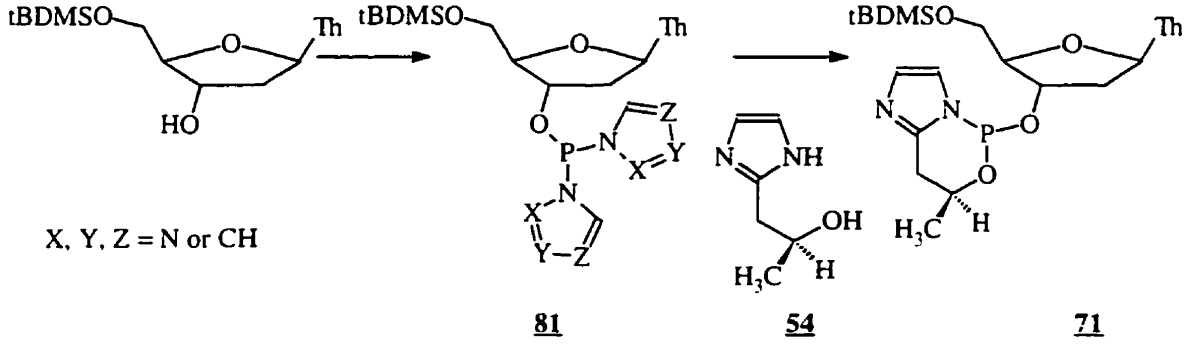
[170] Cogne, A.; Guimaraes, A. G.; Martin, J.; Nardin, R.; Robert, J. B.; Stec, W. J. *Org. Magn. Reson.* **1974**, *6*, 629

[171] Hudson, R. F.; Verkade, J. G. *Tetrahedron Lett.* **1975**, *37*, 3231

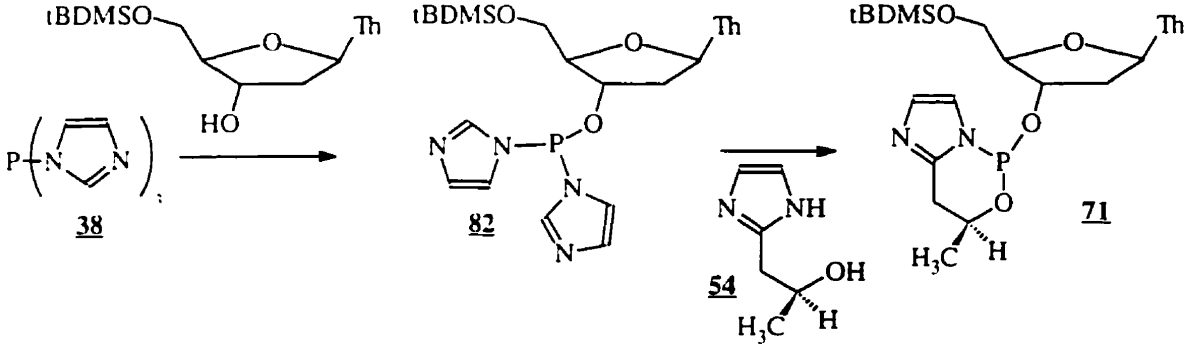
2.4.2.2. Imidazole as a leaving group

As outlined in scheme 2.21, azoles were then considered as potential leaving



Scheme 2.21: the possible use of azoles as leaving groups

groups at the trivalent phosphorus center. Tetrazole, triazole and imidazole had already been used as very labile groups on a trivalent phosphorus atom, as described in scheme 2.1. Tetrazole and triazole were ruled out, given their respective pK_a and nucleophilicity. Imidazole was retained as a good choice. Tri(imidazol-1-yl) phosphine **38** had been used as a good phosphitylating reagent in several cases, and it was used in some instances as a precursor in the synthesis of RNA. It reacted very quickly with 1,2- and 1,3-diols to yield the corresponding cyclic phosphites (the reaction took less than 5 min. at -78°C). Consequently, the strategy presented in scheme 2.22 was considered. We planned to react

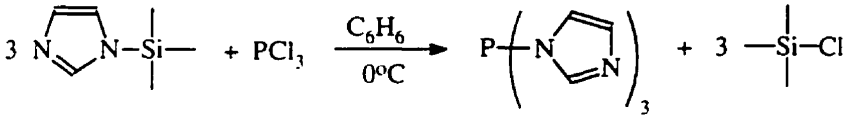


Scheme 2.22: functionalization via tri(imidazol-1-yl) phosphine **38**

chemoselectively tri(imidazol-1-yl) phosphine **38** with 5'-O-tBDMS-thymidine to yield bis-imidazolide **82**. Both remaining imidazole groups would then be displaced by imidazolylpropanol **54** to yield imidazo-oxazaphosphorine **71**. We expected the imidazole group of imidazolylpropanol **54** to benefit from an entropic advantage and displace the

imidazole group of bis-azolide **82**. Tri(imidazol-1-yl) phosphine **38** was first synthesized according to the procedure published by Shimidzu *et al.*[172] by reaction of 6 eq. of imidazole with phosphorus trichloride in THF at 0°C, followed by filtration of the mixture to remove the three equivalents of imidazolium chloride generated. The resulting crude turbid solution can typically be kept for one to two days at ambient temperature under argon[172]. Evaporation of the solvent *in vacuo* led to a thick oil containing a mixture of products, showing ^{31}P NMR signals between 0 and 20 ppm. Given that the exact concentration of the solution was not known and due to its turbid nature, we decided first to improve the synthesis of tri(imidazol-1-yl) phosphine **38**.

By reacting 1-trimethylsilylimidazole **83**, obtained from the reaction of imidazole with neat hexamethyldisilazane (HMDS) at 100°C, with phosphorus trichloride in benzene at 0°C under argon, a white suspension was obtained (scheme 2.23). After evaporation of



83 **38**

Scheme 2.23: new synthesis of tri(imidazol-1-yl) phosphine

the solvent *in vacuo*, a highly reactive white solid was isolated. Upon opening of the reaction flask it burnt in the air immediately. However, when handled under the more rigorous dry conditions, it was possible to analyze it by ^{31}P, ^{1}H, ^{13}C NMR and MS, which all confirmed the structure and purity of the desired product. It could also be handled in an argon atmosphere, and further reactions could be performed. This new way of synthesizing **38** turned out to be more convenient than the procedure described by Shimidzu *et al.*[172] Both the solvent (benzene) and side-product (chlorotrimethylsilane) were removed at the end of the synthesis, leaving a known amount of pure tri(imidazol-1-yl) phosphine **38** as the sole product. Phosphine **38** was poorly soluble in most organic solvents, and a turbid solution was obtained in many instances. The best solvent to solubilize it was dry pyridine.

Chemoselective displacement of a single imidazole group from **38** was more difficult than anticipated. The simultaneous displacement of two imidazole groups had

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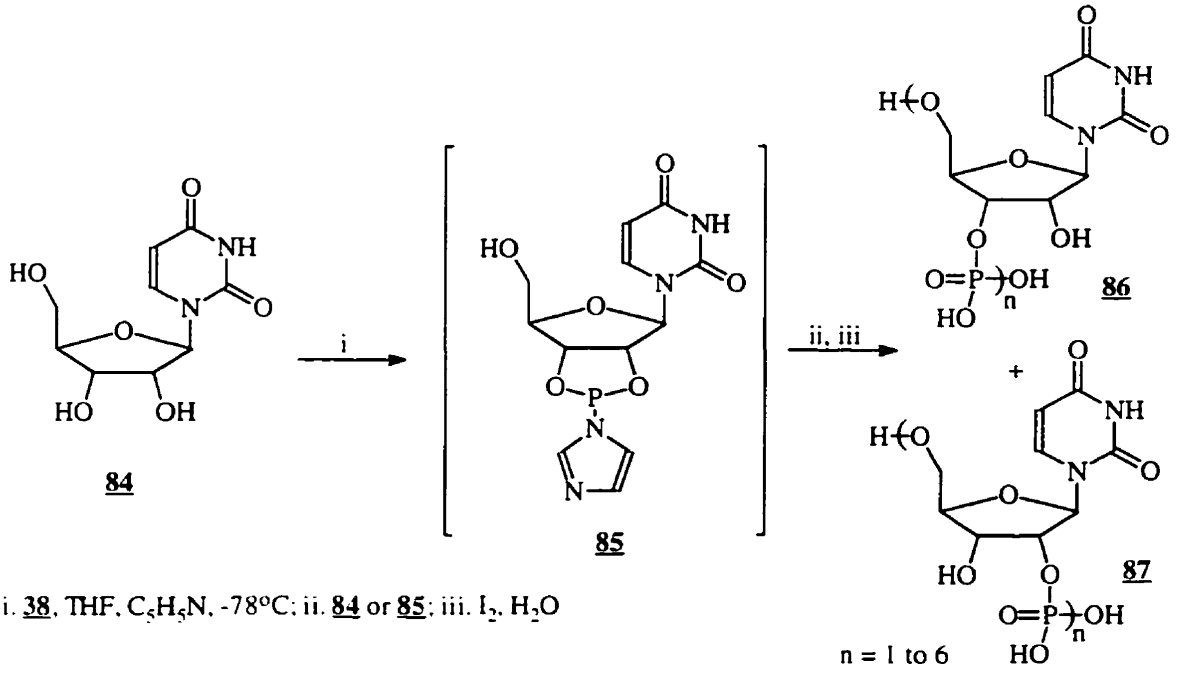
[172] Shimidzu, T.; Yamana, K.; Kanda, N.; Kitagawa, S. *Bull. Chem. Soc. Jpn* **1983**, *56*, 3483

been described as an easy process by Shimidzu *et al.*[173]. When **38** was reacted with 1,2- or 1,3-diols, the reaction occurred within 5 min. at -78°C. As indicated in scheme 2.24, uridine **84** was reacted with tri(imidazol-1-yl) phosphine **38** and led to the initial double displacement by 2'- and 3'-hydroxy groups to yield the intermediate phosphorimidazolide



i. **38**, THF, C_5H_5N, -78°C; ii. **84** or **85**; iii. I_2, H_2O

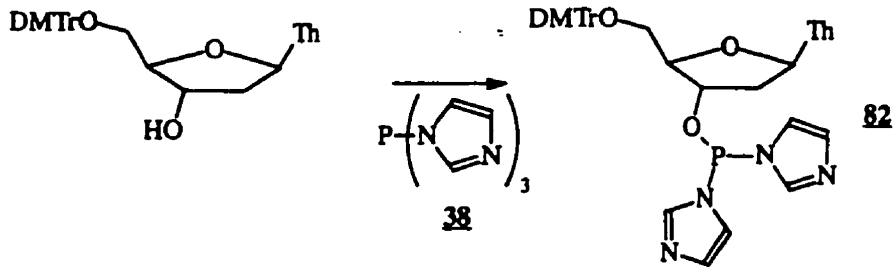
Scheme 2.24: tri(imidazol-1-yl)phosphine **38** as a phosphitylating reagent

85. Eventually, 5'-3' and 5'-2' oligomers **86** and **87** were obtained. The authors then concluded that the formation of the 2',3'-cyclic phosphorimidazolide **85** had to be kinetically controlled and occurred even in the presence of the primary alcohol at the 5' end of uridine.

We started investigating the reaction of a single alcohol with tri(imidazol-1-yl) phosphine **38** in order to synthesize the 3'-activated nucleoside **82** chemoselectively as described in scheme 2.25. First using isopropanol, the displacement was performed on the crude mixture prepared according to Shimidzu *et al.* It led to a mixture of monosubstituted

[173] Shimidzu, T.; Yamana, K.; Murakami, A.; Nakamichi, K. *Tetrahedron Lett.* **1980**, *21*, 2717

product 88, disubstituted product 89 and starting materials 38 after the addition of 1 eq. of



Scheme 2.25: reaction of 5'-O-DMTR-thymidine and tri(imidazol-1-yl)phosphine

alcohol at low temperature in THF. The reaction was repeated with several solvents and temperatures, using our crystalline tri(imidazol-1-yl) phosphine that allowed for the variation of the solvent, as reported in figure 2.7, scheme 2.26 and table 3.

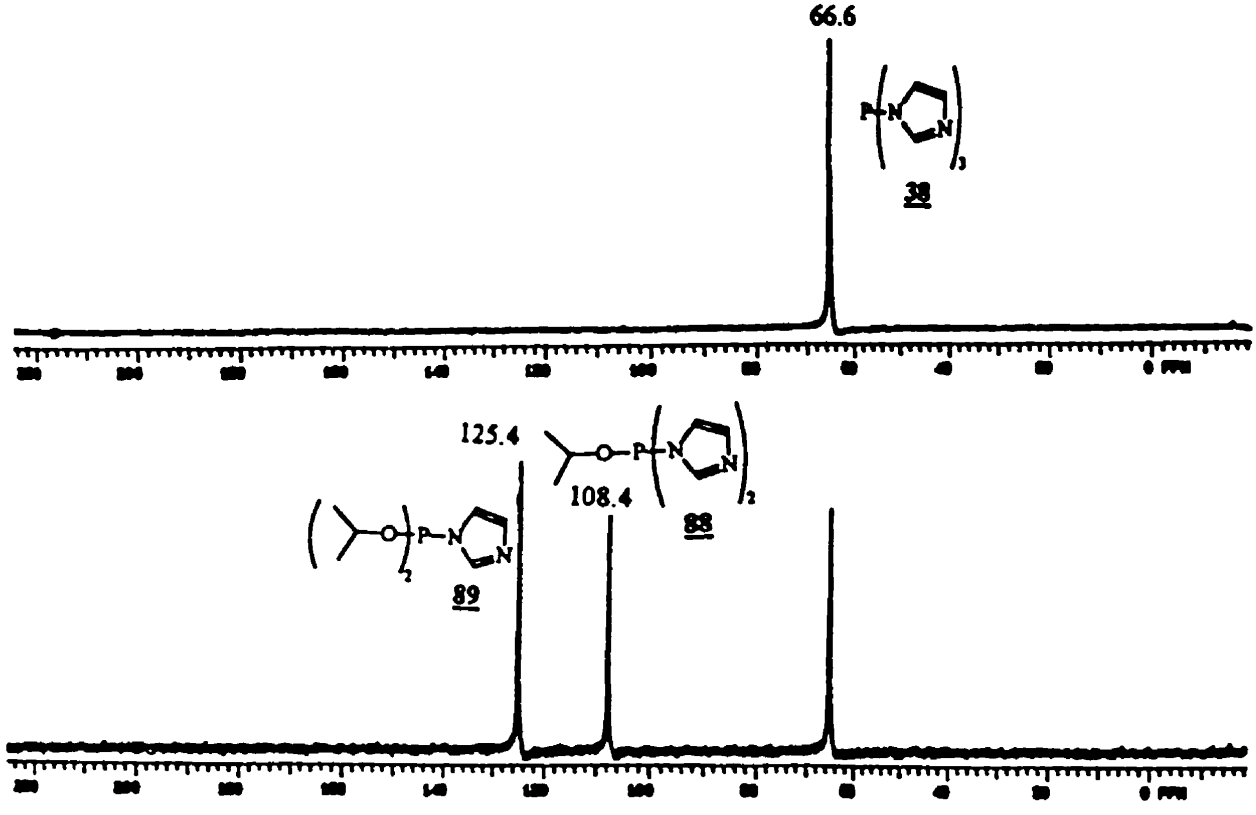
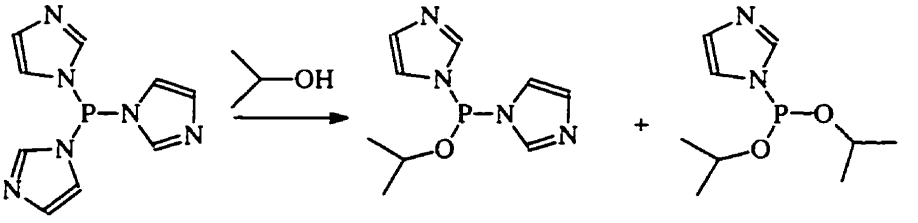


Figure 2.7: ^{31}P NMR spectra of tri(imidazol-1-yl) phosphine 38 (top) and its reaction products 88 and 89 with isopropanol (bottom)



38 δ 66.6 ppm **88** δ 108.4 ppm **89** δ 125.4 ppm

Scheme 2.26: polysubstitution of imidazole moieties

Table 3: variation of the ratio of products with solvent and temperature (1h addition)

Solvent	Temperature (°C)	Ratio **88**:**89**:**38**[a]
dichloromethane	-78	50:25:25
pyridine	-40[b]	30:35:35
pyridine	-20	40:30:30
acetonitrile	-40[b]	40:30:30
acetonitrile	-20	60:20:20
acetonitrile[c]	-20	70:10:10

[a] as evaluated by the relative integration of the ^{31}P NMR signals

[b] pyridine and acetonitrile freeze at -42°C and -48°C respectively

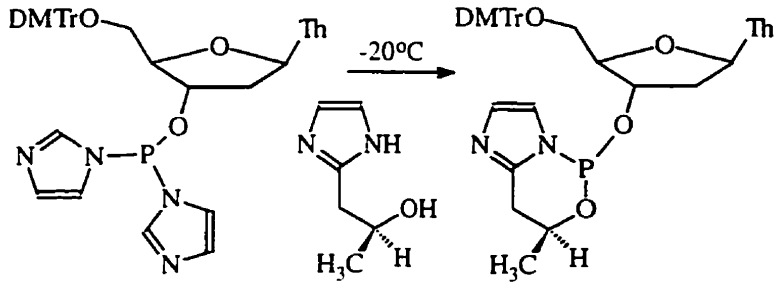
[c] in this case, 5'-O-DMTr-thymidine was used as a nucleophile

Triazolide **38** was very reactive towards nucleophilic substitution and gave the monosubstituted intermediate **88**. The latter was in turn also very susceptible to substitution to yield the disubstituted product **89**. Using only 1eq. of alcohol, the trisubstituted product was not observed. It started to appear when more than 1.5 eq. alcohol was used (δ 140 ppm).

Using acetonitrile as a solvent at -20°C, the displacement was then performed with 5'-O-DMTr-thymidine as a nucleophile. Increasing the length of addition of the alcohol to 15h (as a solution in acetonitrile:dichloromethane 1:1) and using only 0.9 eq. of the latter with respect to tri(imidazol-1-yl) phosphine **38**, made it possible to obtain a ratio of **88**:**89**:**38** as high as 70:10:10, which was the best result obtained in this study. The use of 5'-O-DMTr-thymidine resulted in the formation of less disubstitution product than

isopropanol under identical conditions. This observation was attributed to the greater steric demand of 5'-O-DMTr-thymidine.

On the previously described mixture containing azolides **90**, **38** and the bis-substitution product in a ratio of 70:10:10, imidazolylpropanol **54** was added as a solution in pyridine at -20°C (scheme 2.27). The resulting **77** was obtained as a mixture of



90
δ 107.4 ppm

54

77
δ 118.6 ppm (major)
δ 119.0 ppm (minor)
ratio3:1

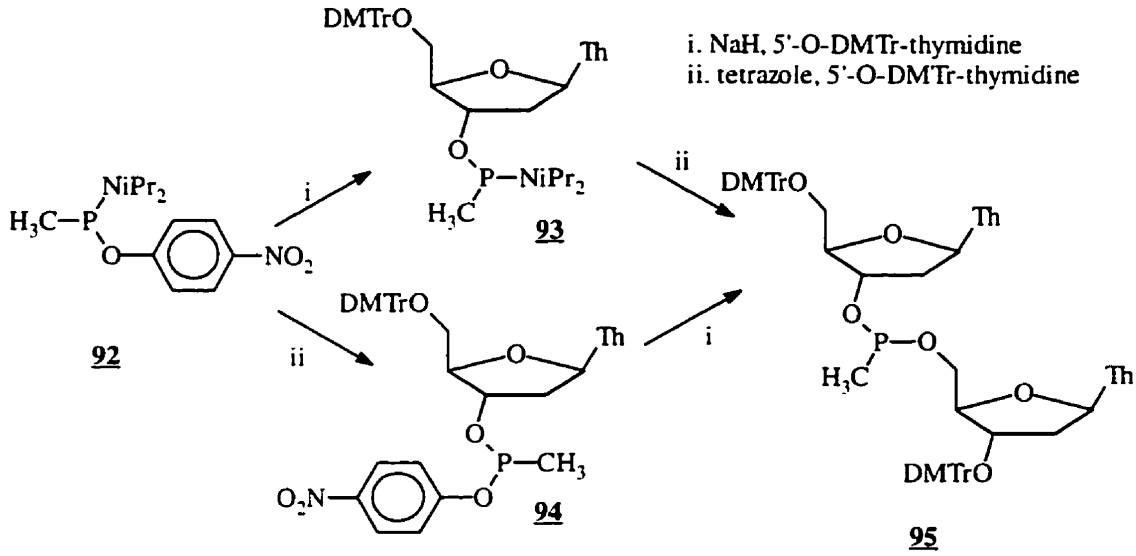
Scheme 2.27: introduction of imidazolylpropanol **54**

diastereomers in a ratio of 1:3 appearing at 119.0 and 118.6 ppm respectively, as established by ^{31}P NMR. A few side-products arising from the reaction of imidazolylpropanol **54** with triimidazolylphosphine **38** and its disubstituted derivative **89** were also observed. Heating the solution to 40°C did not improve the diastereomeric ratio.

From this part of the work, it became clear that tri(imidazol-1-yl) phosphine **38** did not undergo a single substitution by an alcohol. The results were even worse when a primary alcohol such as 3'-O-tBDMS-thymidine was used as a nucleophile, in which case the amount of disubstituted product was larger than the amount of monosubstituted one, no matter which conditions were used. Besides, in the only experiment that led to imidazo-oxazaphosphorine **77**, the latter appeared as a 3:1 mixture of diastereomers plus side-products. Consequently, we did not continue in this direction and turned our efforts towards the use of another type of leaving group that might allow for the isolation of the "3'-activated" nucleoside.

2.4.2.3. Substituted phenols as leaving groups

In 1991, Helinski *et al.*[174] introduced the *p*-nitrophenoxy group as a good leaving



Scheme 2.28: the use of bifunctional phosphonamidites

group from a trivalent phosphorus atom in basic conditions. As indicated in scheme 2.28, the originality of their approach lay in its versatility. Using phosphonamidite **92** that could be activated selectively in basic or acidic conditions, they were able to synthesize intermediates **93** and **94**. Intermediates **93** and **94** then reacted in acidic or basic conditions respectively to yield the same product methylphosphonite **95**, which was subsequently oxidized to the corresponding methylphosphonate or sulfurized to the methylthiophosphonate dinucleotide. Replacing the methyl group on precursor **92** by a second *p*-nitrophenoxy group[175] allowed them to synthesize phosphorodithioate and phosphoroselenothioate dimers of thymidine. Replacing the methyl group on **92** by a methoxy group, and sodium hydride by DBU[176], led to the preparation of phosphorothioate and phosphoroselenoate dimers, as well as mixed phosphorothioate and phosphoroselenoate trimers.

We considered this strategy as a possible route to intermediate imidazo-oxazaphosphorine **91**, as outlined in scheme 2.29, in which an activated phosphite triester bearing two *p*-nitrophenoxy groups **96** would be formed. The two *p*-nitrophenoxy groups

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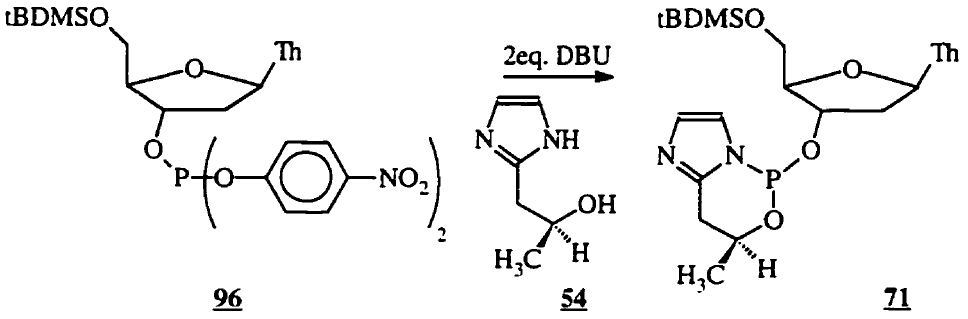
[174] Helinski, J.; Dabkowski, W.; Michalski, J. *Tetrahedron Lett.* **1991**, *32*, 4981
[175] Helinski, J.; Dabkowski, W.; Michalski, J. *Tetrahedron Lett.* **1993**, *34*, 6451
[176] Helinski, J.; Dabkowski, W.; Michalski, J. *Nucleosides Nucleotides* **1993**, *12*, 597

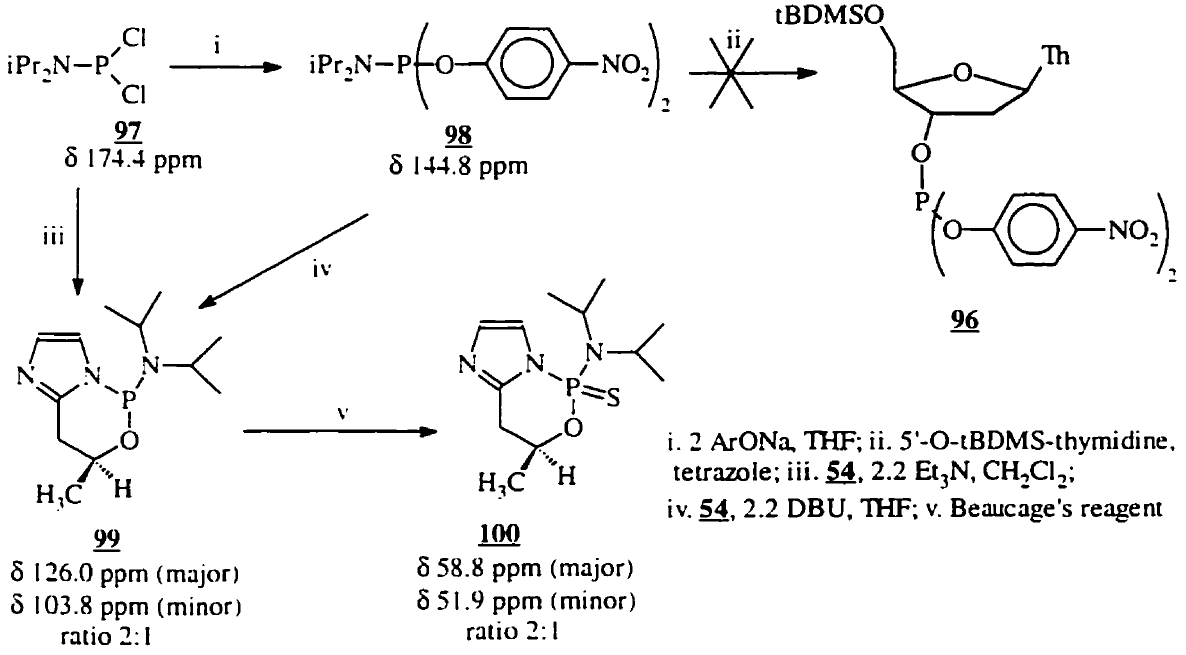
would subsequently be displaced simultaneously by imidazolylpropanol **54** to yield the



Scheme 2.29: use of a *p*-nitrophenoxy group

desired imidazo-oxazaphosphorine **71**.

Therefore N,N-diisopropylamino-bis-(4-nitrophenoxy)phosphoramidite **98** was synthesized by reaction of sodium 4-nitrophenoxide on diisopropylaminophosphoramidous dichloride in dry THF according to the procedure described by Helinski *et al.*[176]. However, in the presence of 5'-O-tBDMS-thymidine and tetrazole, phosphoramidite **98** did not react as indicated in scheme 2.30 to give phosphite triester **96**. Using the stronger acid 2-bromo-

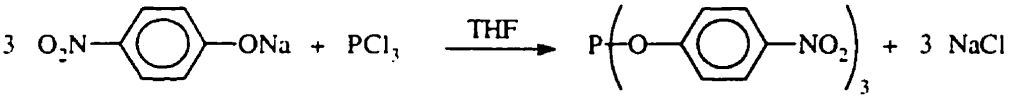


Scheme 2.30: the use of N,N-diisopropyl bis(4-nitrophenyl) phosphoramidite

4,5-dicyanoimidazole did not lead to any reaction either. This reaction was not described by Helinski *et al.*, who reacted precursor **98** with a nucleoside in the presence of DBU, not

in acidic conditions. The presence of a single *p*-nitrophenoxy group on the phosphorus atom allowed for the protonation of the diisopropylamino moiety by tetrazole as described by Helinski *et al.*[174-176]. However, the presence of a second *p*-nitrophenoxy group did not allow this activation, presumably due to the lower electron density which decreased the basicity of the nitrogen atom. The reaction of phosphoramidite **98** with imidazolylpropanol **54** was then performed, in order to evaluate whether the imidazole group would be able to substitute the *p*-nitrophenoxy group. The reaction occurred within 30 min., and two diastereomers of imidazo-oxazaphosphorine **99** were obtained in a ratio of 2:1, having resonance frequencies at 126.0 and 103.8 ppm respectively. This mixture was then sulfurized using Beaucage's reagent, and both diastereomers of the corresponding thio adduct **100** were observed at 58.8 and 51.9 ppm in the same ratio. They could be partially separated by flash chromatography and characterized by ^{1}H, ^{13}C, ^{31}P NMR and M.S. This sequence of reactions was confirmed by the parallel reaction of imidazolylpropanol **54** with diisopropylphosphoramidous dichloride **97** in the presence of triethylamine, giving the same result. Similarly to the reaction of **54** with HMPT yielding both diastereomers of the corresponding imidazo-oxazaphosphorine **78** (section 2.4.2), two diastereomers were again obtained. Appendix 2 shows the ^{1}H NMR spectrum of heterobicyclic **100**.

Knowing that a nucleoside moiety could not be introduced by acid-catalyzed displacement of the diisopropylamino group of phosphoramidite **98**, the synthesis of phosphite triester **101** was then performed from phosphorus trichloride and three equivalents of sodium *p*-nitrophenoxide as shown in scheme 2.31. The reaction gave a



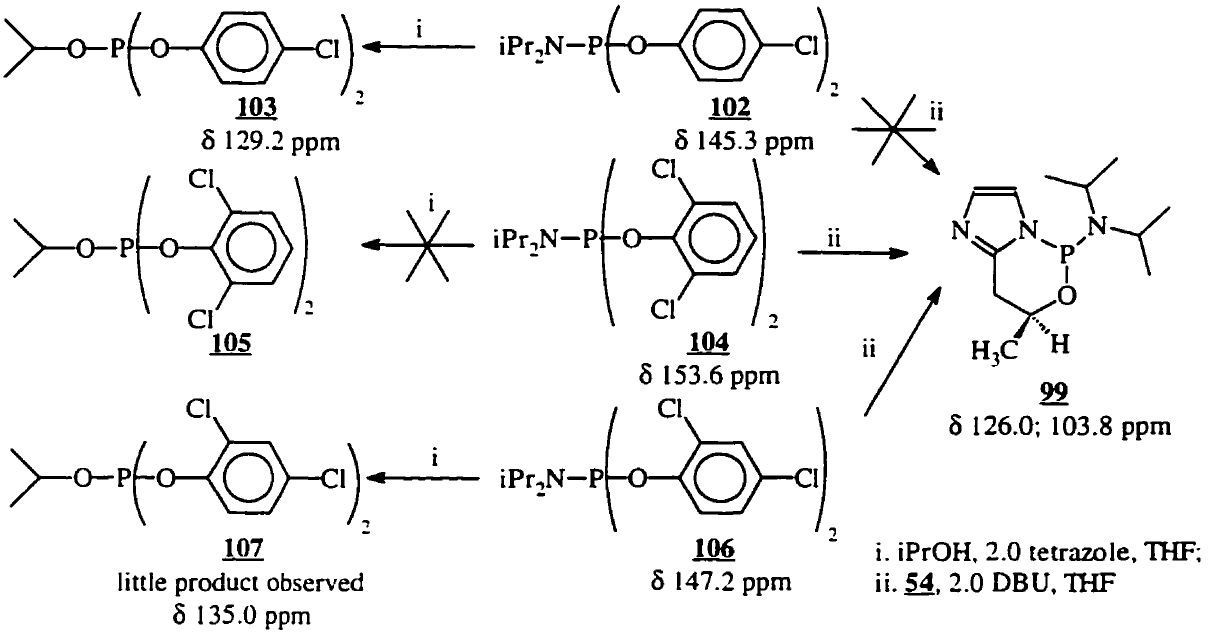
101
δ 125.7 ppm

Scheme 2.31: synthesis of tri(4-nitrophenyl) phosphite

compound having a ^{31}P NMR resonance signal at 125.7 ppm. However this compound could not be purified properly and decomposed quickly upon standing. Other substituted phenoxides were then considered, in order to find the one that would be sufficiently stable

to be isolated, yet reactive enough to be susceptible to substitution by a nucleoside, then by imidazolylpropanol **54**.

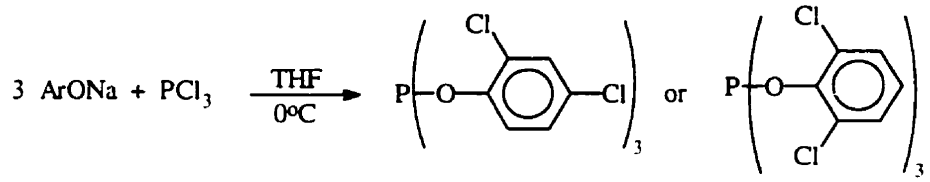
The simultaneous nucleophilic displacement by both groups of **54** was attempted on several substituted arylphosphoramidites as depicted in scheme 2.32. The *p*-



Scheme 2.32: the displacement of various phenoxides

chlorophenoxy group proved to be a poor leaving group and could not be displaced on phosphoramidite **102** by imidazolylpropanol **54** (scheme 2.32). On the other hand, this group did not decrease the basicity of the diisopropylamino moiety on **102** too much, so that the acid-catalyzed displacement of the *p*-chlorophenoxy moiety yielded phosphite triester **103** having a resonance signal at 129.2 ppm. Both the 2,6- and 2,4-dichlorophenoxy groups were good leaving groups on phosphoramidites **104** and **106** respectively. Both of them underwent a DBU-catalyzed displacement and bicyclic imidazo-oxazaphosphorine **99** was obtained in both cases, with a reaction time lower than 30 min. However, the 2,6-dichlorophenoxy moiety did not allow the acid-catalyzed displacement of the diisopropylamino group, while the 2,4-dichlorophenoxy did allow it to a small extent after 3h reaction time. This difference was attributed to the difference in steric hindrance in these two groups, the presence of a 6-chloro group making it more difficult for the amino group to be protonated.

Knowing that 2,4- and 2,6-dichlorophenoxy were good leaving groups at the trivalent phosphorus atom, activated phosphite triesters **108** and **109** were then synthesized (scheme 2.33), from the reaction of the corresponding sodium phenoxide salt



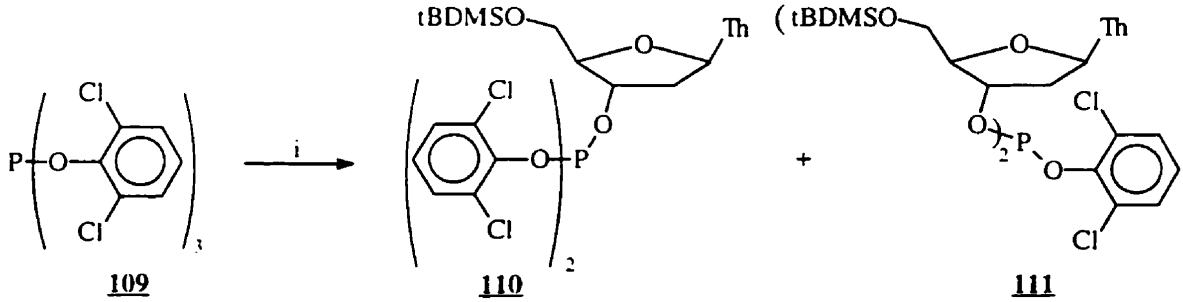
Ar = 2,4-dichlorophenyl or 2,6-dichlorophenyl

108 δ 129.0 ppm

109 δ 141.7 ppm

Scheme 2.33: synthesis of tri(aryl) phosphites

and phosphorus trichloride. Phosphite triester **109** could be isolated and purified by filtration on a short pad of silica gel, whereas its structural isomer **108** underwent some decomposition under the same conditions. There again, this higher sensitivity of triester **108** as compared to **109** towards hydrolysis, was attributed to the smaller steric hindrance around the phosphorus atom in **108**. Therefore this study was continued using reactive phosphite triester **109**. The latter was reacted with 5'-O-tBDMS-thymidine in THF, in the presence of 1.0 eq. DBU. The reaction was not chemoselective, the desired mono-substituted product **110** (δ 131.3 ppm) as well as the product of double condensation **111** (δ 137.6 ppm) being obtained in a ratio of 7:3 (scheme 2.34). This ratio remained



109 δ 141.7 ppm

110 δ 131.3 ppm

111 δ 137.6 ppm

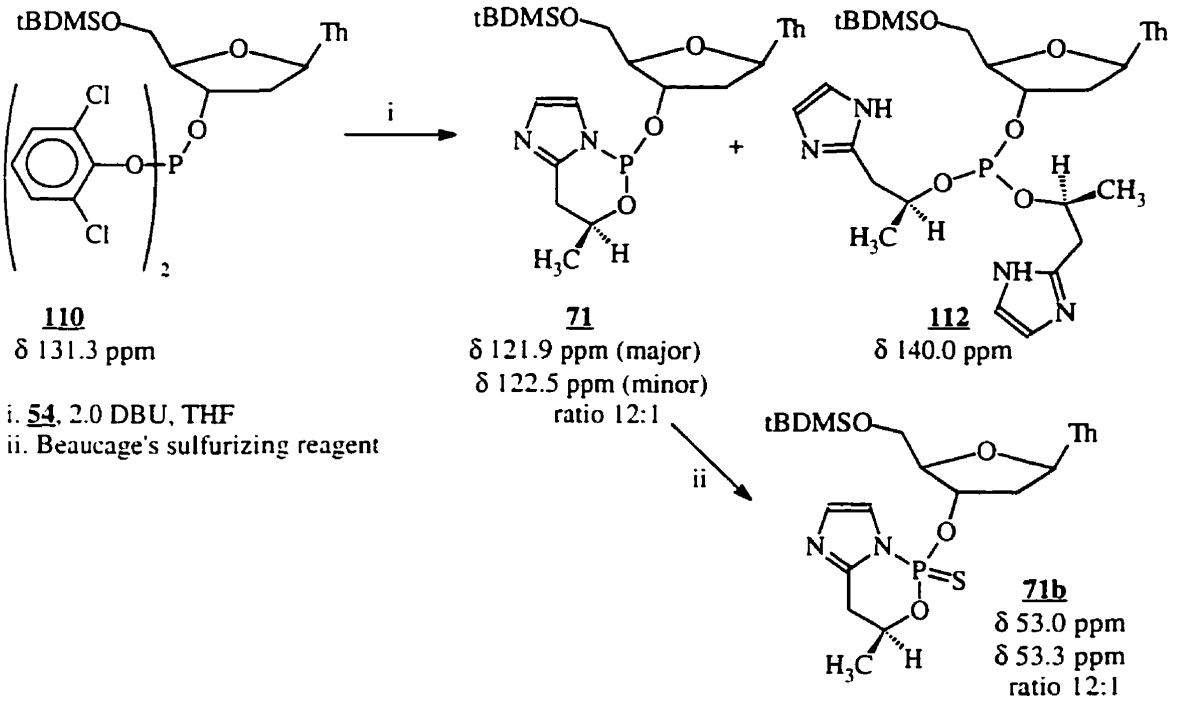
i. 5'-O-tBDMS-thymidine, DBU, THF

Scheme 2.34: substitution of dichlorophenol by 5'-O-tBDMS-thymidine

identical when the solvent was changed from THF to chloroform, and when the order of introduction of the reagents was modified. Both compounds could be separated by flash

chromatography and **110** could be obtained pure in 65% yield. It was characterized by ^{31}P, ^{1}H, ^{13}C NMR and MS. In the absence of DBU, no reaction took place. Thus, in pyridine or in the presence of 5 eq. of triethylamine, no trace of reaction was observed after 4h. Appendix 3 shows the ^{1}H NMR spectrum of triester **110**, appendix 4 its 2D-COSY map.

The next step was the DBU-catalyzed double displacement of both dichlorophenoxy groups from phosphite triester **110** by imidazolylpropanol **54**. Two types of products were obtained in a ratio of 1.2:1, the first set corresponding to both diaste-



Scheme 2.35: double substitution of dichlorophenol by imidazolylpropanol

reomers of imidazo-oxazaphosphorine **71** in a ratio of 12:1, the second set to phosphite triester **112**, derived from the reaction of bicyclic **71** with **54** (scheme 2.35 and figure 2.8). The formation of phosphite triester **112** was not observed during the synthesis of imidazo-oxazaphosphorine **99** from activated phosphite triesters **98**, **104** and **106** (schemes 2.30 and 2.32). This difference in reactivity was attributed to the presence of a more electron-withdrawing alkoxy group on **71**, as opposed to an amino group on **99**, which consequently would make the phosphorus atom less prone to nucleophilic substitution. Varying the solvent (pyridine, chloroform), the order of introduction of the reagents and the temperature did not improve the results. Changing the temperature from ambient to

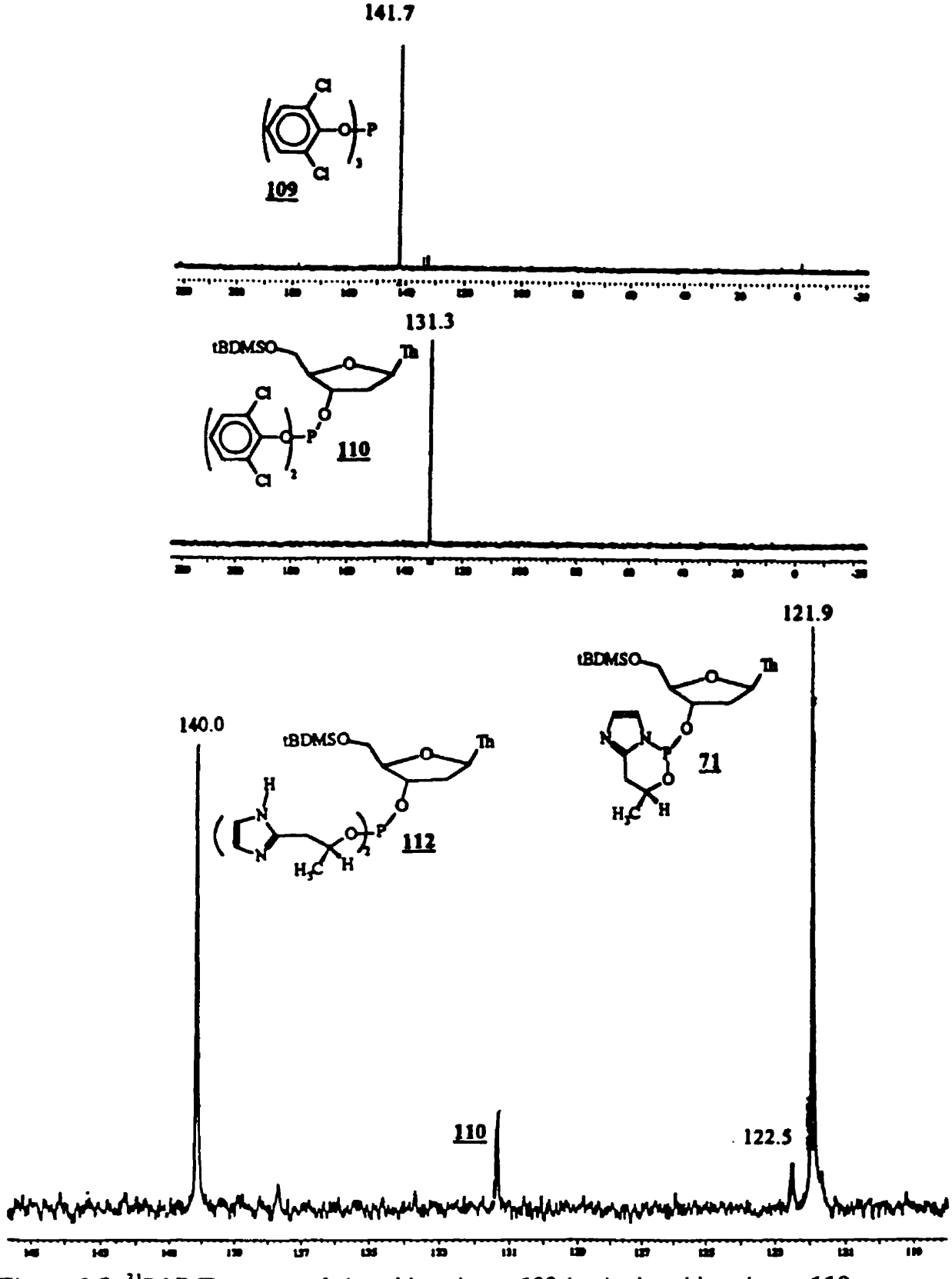


Figure 2.8: ^{31}P NMR spectra of phosphite triester **109** (top), phosphite triester **110** (center, after purification) and its reaction products with **54** (bottom)

-40°C resulted in a change of the ratio **71**:**112** from 1.2:1 to 1:2, leading to the conclusion that **71** was more reactive than **110** towards nucleophilic substitution. There again, it appeared that the conditions used were too aggressive for the highly reactive imidazo-oxazaphosphorine **71**. Here, Beaucage's reagent was added to the reaction mixture and after column chromatography, the major diastereomer of the resulting imidazo-oxazaphosphorine **71b** could be obtained and characterized by ^{1}H, ^{13}C, ^{31}P NMR and MS.

In conclusion, imidazolylpropanol **54** is a good chiral precursor for the synthesis of simple phosphite triesters and the corresponding phosphorothioate triesters. In principle, it should also allow the synthesis of chiral phosphoroselenoates and boranophosphates since the difference in the elaboration of these products resides in the last step, after the stereochemistry has been installed. However, the major limitation of this precursor lies in its high reactivity, which makes its subsequent isolation impossible at the trivalent phosphorus stage. This limited the use of imidazo-oxazaphosphorine **56** to the *in situ* generation of the intermediate. However, this *in situ* generation turned out to be, to the best of our knowledge and skills, extremely difficult to carry out when a nucleoside had to be used as the exocyclic phosphorus substituent.

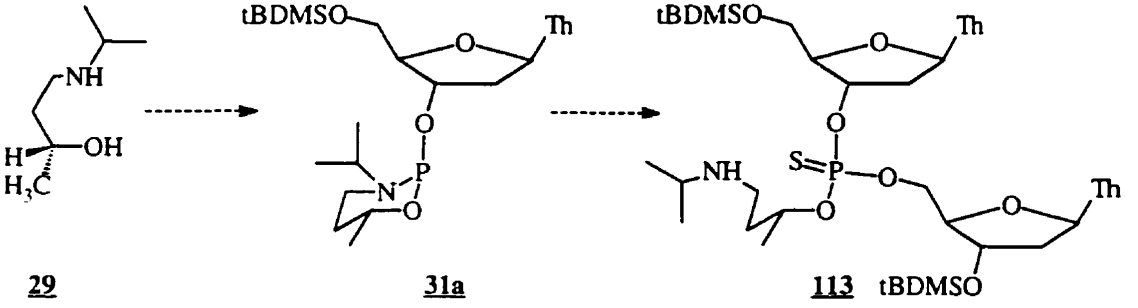
This study led to the conclusion that the intermediate should be less labile, and the indole group was considered as a new potential leaving group. This new orientation is now being investigated by Mr. Jianchao Wang in our laboratory[177].

[177] Wang, J.; Just, G. *manuscript in preparation*

3. New oxazaphosphorinanes incorporating a tertiary alcohol

3.1. Introduction

In parallel to the studies described in section 2 aiming at using imidazo-oxazaphosphorines as precursors to chiral phosphite triesters, the work from Mrs. Zhili Xin[148.149] (scheme 3.1) gave rise to another research direction in our laboratory.



29 **31a** **113**

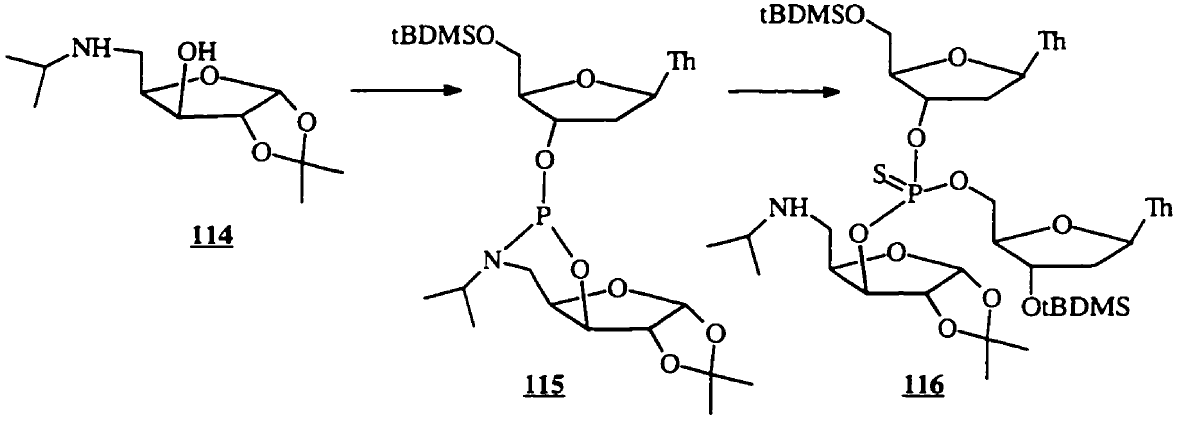
Scheme 3.1: Xin and Just's use of oxazaphosphorinanes

In order to make the method established by Xin and Just applicable to the diastereoselective synthesis of DNA phosphorothioates, two major requirements had to be met. The diastereoselectivity of the coupling step had to be improved and the precursor γ-aminoalcohol **29** had to be transformed into an analogue that would be removable at the end of the synthesis.

The first requirement was initially investigated by Mr. G. Biancotto, who used chiral γ-aminoalcohol **114** as a precursor[178] (scheme 3.2). Chiral auxiliary **114**, synthesized from 1,2-O-isopropylidene-D-xylofuranose, was reacted with phosphorus trichloride and then 5'-O-tBDMS-thymidine to yield chiral oxazaphosphorinane **115** as a single diastereomer, which was stable to chromatography on silica gel. Reaction of **115** with 3'-O-tBDMS-thymidine in the presence of 2 eq. of 2-bromo-4,5-dicyanoimidazole as a catalyst in dry acetonitrile led to the desired chiral phosphite triester in a diastereomeric ratio of 1:6. Subsequent sulfurization yielded phosphorothioate triester **116** in the same ratio. However, removal of the 1,2-O-isopropylidene group from the latter required

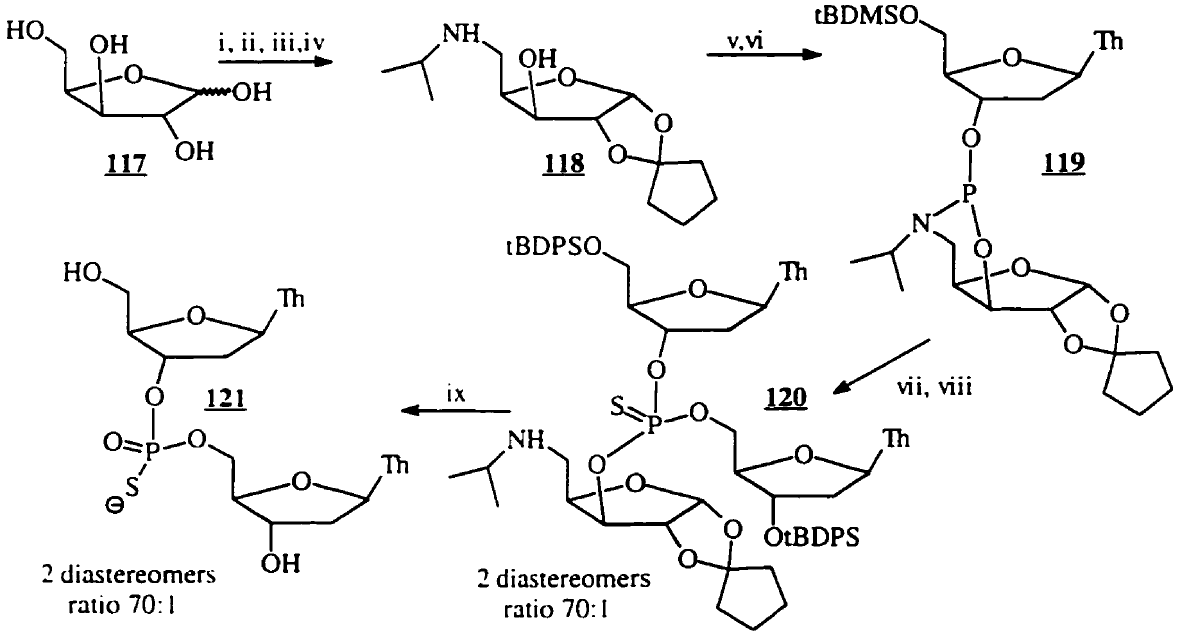
[178] Jin, Y.; Biancotto, G.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 973

strongly acidic conditions, that were incompatible with the presence of pyrimidine or purine bases.



Scheme 3.2: Biancotto and Just's chiral auxiliary

Following this new approach (scheme 3.3), Mrs. Yi Jin modified the starting aminoalcohol by replacing the 1,2-O-isopropylidene protective group by the more



i. cyclopentanone, H^+, $Me_2C(OMe)_2$; ii. AcOH; iii. TsCl, C_5H_5N; iv. $iPrNH_2$, neat, 80°C, P;
v. PCl_3, 2 Et_3N, CH_2Cl_2; vi. 5'-O-tBDPS-thymidine, Et_3N;
vii. 3'-O-tBDPS-thymidine, 2-bromo-4,5-dicyanoimidazole, $CHCl_3$, -15°C;
viii. Beaucage's reagent; ix. 70% TFA, RT

Scheme 3.3: Jin and Just's chiral auxiliary

acid labile 1,2-O-cyclopentylidene group[178]. Starting from D-xylose, chiral γ-aminoalcohol **118** was synthesized in four steps. This precursor was subsequently reacted with phosphorus trichloride in the presence of 2 eq. triethylamine, then with 5'-O-tBDPS-thymidine to yield cyclic oxazaphosphorinane **119** which could be purified chromatographically. Upon reaction of **119** with 3'-O-tBDPS-thymidine, they obtained the corresponding phosphite triester in ratios as high as 70:1 after optimization of the coupling conditions. In order to achieve this high diastereoselectivity, acetonitrile had to be replaced by chloroform as a solvent, and the temperature reduced around 0 to -15°C, increasing the reaction time to several hours. Subsequent sulfurization yielded phosphorothioate triester **120** in the same diastereomeric ratio. The removal of the chiral auxiliary required reaction in 70% trifluoroacetic acid for several hours. The present dinucleoside was stable enough to survive these conditions, and fully deprotected phosphorothioate dimer **121** was obtained as a mixture of diastereomers in a ratio of 70:1. The assignment of its configuration is currently being determined by HPLC analysis and Snake venom phosphodiesterase digestion. In the same reaction conditions, starting from L-xylose, Mrs. Yi Jin obtained the opposite diastereoselectivity in comparable ratios.

These experiments definitely proved that chiral phosphite triesters could be synthesized in high diastereomeric excess by using chiral oxazaphosphorinanes as precursors to orient the stereochemistry at the phosphorus atom. Simultaneously, 2-bromo-4,5-dicyanoimidazole was required as a catalyst. However, the conditions employed here were suitable to solution synthesis but had to be optimized further to become applicable to solid phase synthesis. The coupling temperature had to be closer to ambient temperature, and the coupling time had to be reduced. Similarly, the deprotection conditions (70% TFA for several hours) were on the edge of stability for nucleosides.

In order to address this last requirement, we thought of taking advantage of the leaving ability of the phosphorothioate group. It is well known[179] (scheme 3.4) that a leaving group located on a tertiary carbon atom like **122** has a good ability to depart in order to form the corresponding carbonium ion **123** because the latter is stabilized by

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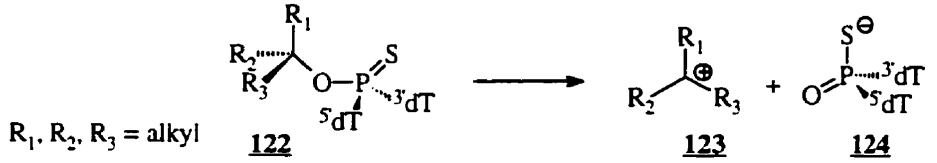
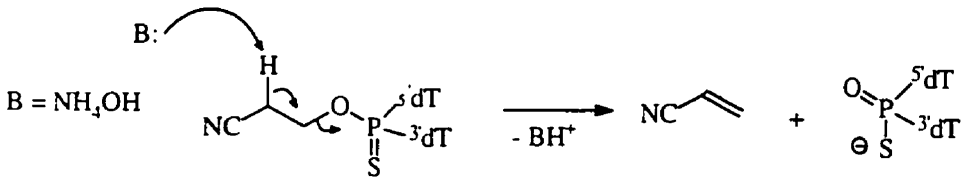
[179] March, J., "*Advanced Organic Chemistry, 4th ed.*" **1992**, John Wiley and Sons Eds.; pp166-173

electron donating effects from the alkyl groups. We considered this property as an



Scheme 3.4: the use of a carbocation formation

alternative to the strong deprotection conditions used previously. The leaving ability of the phosphorothioate group **124** has already been demonstrated in the phosphoramidite approach. The last step that allows the release of the phosphorothioate moiety is based on a β-elimination relying on two effects. The acidity of the proton at the α position to the cyano group, and the leaving ability of the phosphorothioate group make this process possible with a weak base such as ammonium hydroxide (scheme 3.5), in a short time.



Scheme 3.5: the deprotection of nucleotide at the end of the phosphoramidite cycle

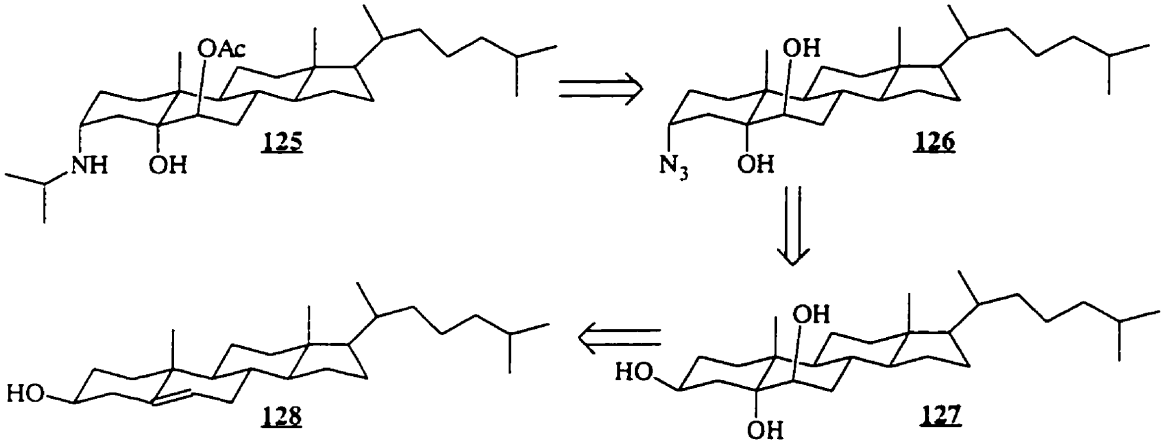
The synthesis of a chiral γ-aminoalcohol in which the alcohol was tertiary was therefore considered. This chiral precursor also had to be appreciably sterically hindered in order to favor one diastereomer over the other during the coupling step.

Natural products offer a variety of attractive compounds which all are available in a very high chiral purity. Considering this array of possible precursors, two types of chiral auxiliaries were chosen regarding the present issue. The first one possessed a steroid backbone derived from cholesterol, and the second a bicyclic terpene backbone derived from camphor. Both of them featured a very hindered γ-aminoalcohol moiety incorporating a tertiary alcohol, which would create a stabilized carbonium ion upon departure of the phosphorothioate group.

In the following sections 3.2 and 3.3, we shall discuss the results obtained when these two new chiral auxiliaries were investigated as potential precursors.

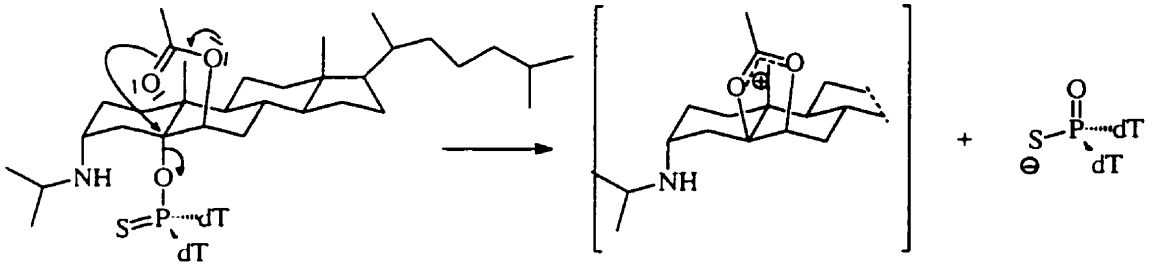
3.2. Use of oxazaphosphorinane 135 derived from cholesterol

The first γ-aminoalcohol **125** that met the requirements presented earlier was derived from cholesterol. It possessed a γ-aminoalcohol moiety incorporating a tertiary alcohol and a *trans* oriented hydroxy group adjacent to it, which, if needed, could assist in the departure of the phosphorothioate if transformed in an acetate or a carbamate. Precursor **125** could be synthesized in six steps from cholesterol with a full control of the stereochemistry (scheme 3.6).



Scheme 3.6: retrosynthetic analysis of chiral auxiliary **125**

The acetate had already been reported[180] in many instances to favor the departure of an adjacent group when located in an *anti* position with respect to the leaving group, as indicated in scheme 3.7.

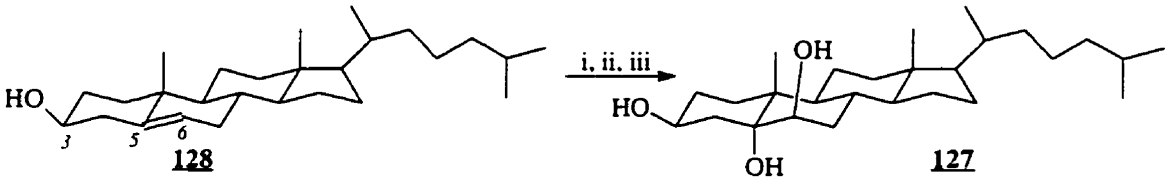


Scheme 3.7: participation of the 6-acetate

[180] Winstein, S.; Hanson, C.; Grunwald, E. *J. Am. Chem. Soc.* **1948**, *70*, 812

3.2.1. Synthesis of chiral γ-aminoalcohol 125 derived from cholesterol

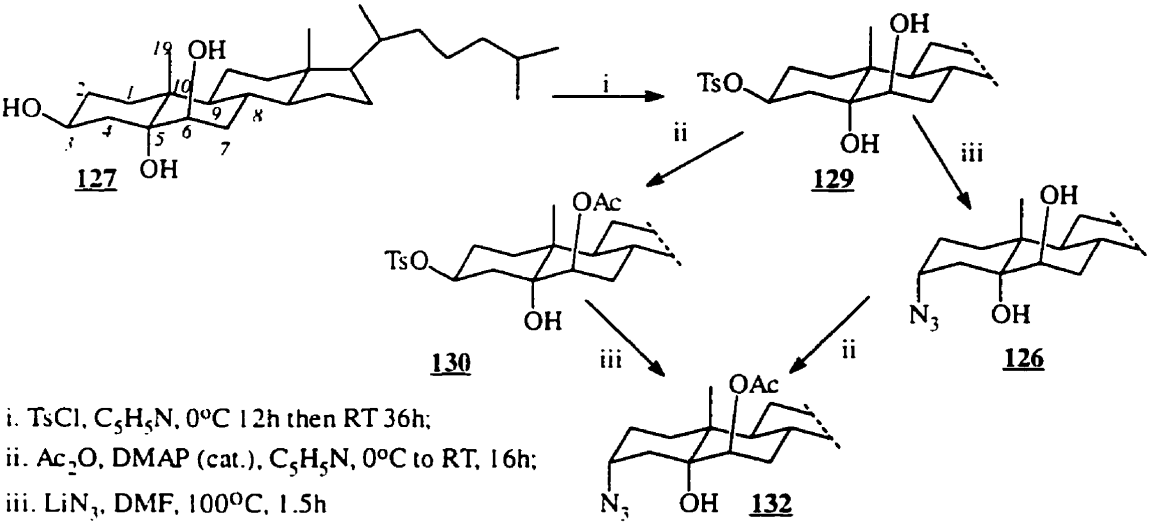
The synthesis started from cheap, commercially available cholesterol **128**, which was first reacted with formic acid[181] at 80°C to form cholesterol formate. To this mixture



i. H_2O_2, HCO_2H; ii. NaOH, CH_3OH; iii. H^+, CH_3OH

Scheme 3.8: the 5,6 *trans* dihydroxylation of cholesterol

cooled down to ambient temperature was added hydrogen peroxide (scheme 3.8), in order to form the epoxide at the 5,6 position. After overnight reaction, the crude product (a mixture of triol **127** and its formate derivatives) was precipitated by adding cold water to the solution. To the crude mixture was added a solution of sodium hydroxide in methanol to saponify the formate groups. It was then acidified and the product was precipitated from water. The white solid thus obtained was recrystallized from methanol.



i. TsCl, C_5H_5N, 0°C 12h then RT 36h;
ii. Ac_2O, DMAP (cat.), C_5H_5N, 0°C to RT, 16h;
iii. LiN_3, DMF, 100°C, 1.5h

Scheme 3.9: installation of the stereochemistry at C_3

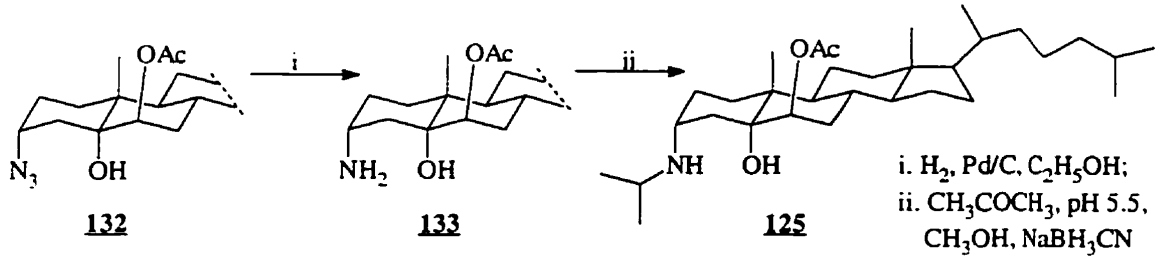
Triol **127** was then regioselectively tosylated at position 3 in 80% yield (scheme 3.9). The equatorial tosyl group of **129** was subsequently displaced by lithium azide in DMF at 100°C. The reaction proceeded in 1.5h, and azide **126** was isolated with no

[181] Fieser, L. F.; Rajagopalan, S. *J. Am. Chem. Soc.* **1949**, *71*, 3938

chromatographic purification in 98% yield, installing the desired stereochemistry at position 3. This intermediate was then acetylated by acetic anhydride in pyridine, catalyzed by 4-dimethylaminopyridine to yield the 6-acetoxy derivative **132** in essentially quantitative yield.

A parallel route was carried out, in which tosylate **129** was first acetylated at hydroxyl 6 to yield acetate **130**, which was then reacted with lithium azide to give azide **132**. The reaction conditions and yields for these transformations were similar to the ones described.

Azide **132** was then catalytically hydrogenated (scheme 3.10) in the presence



132 **133** **125**

Scheme 3.10: reduction and reductive alkylation

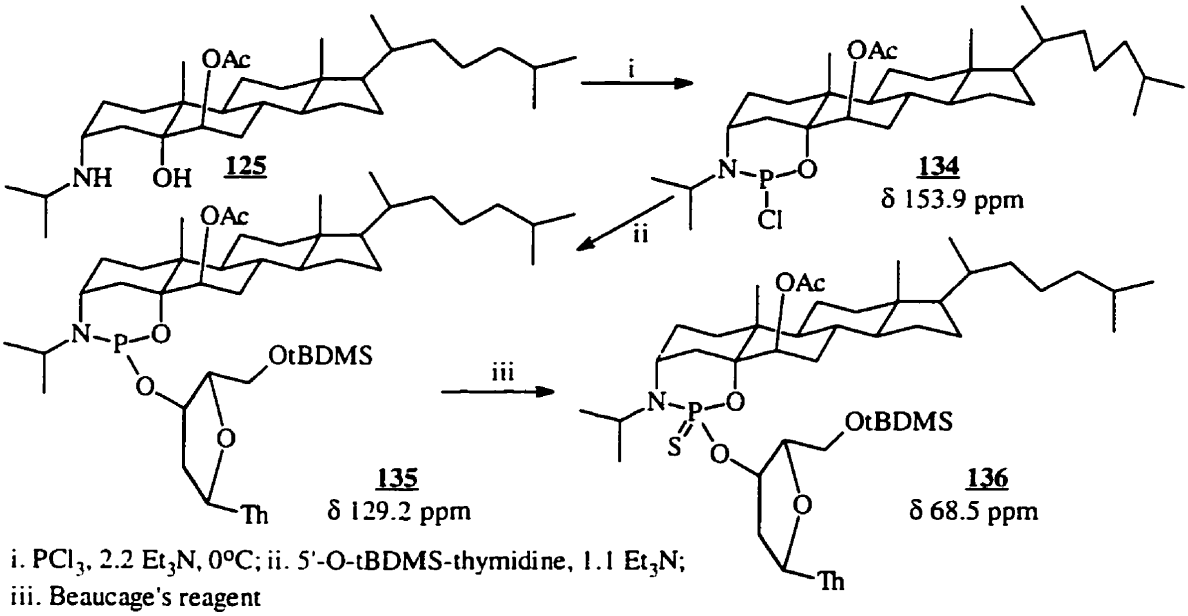
of 10% palladium over charcoal in ethanol. The crude amine **133** was then directly subjected to reductive alkylation with acetone in the presence of sodium cyanoborohydride in methanol at pH 5.5 over 24 h. Pure N-alkylated amine **125** was then obtained after flash chromatography in 72% overall yield starting from azide **132**.

The next step consisted in investigating whether chiral γ-aminoalcohol **125** could be a potential precursor in the diastereocontrolled synthesis of phosphorothioates.

3.2.2. Synthesis of oxazaphosphorinane 135 derived from cholesterol

γ-Aminoalcohol **125** was subjected to condensation with phosphorus trichloride (scheme 3.11) in dichloromethane in the presence of 2.2 eq. of triethylamine at 0°C. Immediately after the mixture of γ-aminoalcohol **125** and triethylamine was added to a solution of phosphorus trichloride in dichloromethane or chloroform at 0°C, a single ^{31}P NMR signal appeared at 153.9 ppm. The reaction occurred very quickly, as opposed to the condensation of chiral γ-aminoalcohols synthesized by Jin and Biancotto, which initially

showed a mixture of compounds slowly evolving to a single product after several hours.



i. PCl_3, 2.2 Et_3N, 0°C; ii. 5'-O-tBDMS-thymidine, 1.1 Et_3N;
iii. Beaucage's reagent

Scheme 3.11: the use of γ-aminoalcohol **125** as a precursor

The faster formation of a single compound with precursor **125** was attributed to an entropic advantage. Aminoalcohol **125** had a rigid structure in which the alcohol and amine functionalities were already pre-organized, no rotation around any of the relevant bonds being allowed, setting the right conformation for double displacement at phosphorus trichloride. Kinetically, this would eliminate several possible conformations present in previous chiral auxiliaries, resulting in a faster formation of the corresponding oxazaphosphorinane **134**.

The second step was performed in the same reaction flask, and also showed a very rapid reaction. Depending on the conditions, one or two products were observed. When the addition of a solution of 5'-O-tBDMS-thymidine and 1.1 eq. of triethylamine in chloroform was carried out slowly at low temperature (-20°C or 0°C), two products **135** were observed in a ratio of 1:5 at 132.3 and 129.2 ppm respectively. Heating the reaction mixture up to 60°C for several hours influenced this ratio very slowly. After 4h heating, it was 1:6. After 24h at 60°C, a significant amount of hydrolyzed products was present. However, when the addition was done quickly at 60°C, only one product was observed, at 129.2 ppm. Figure 3.1 presents the corresponding ^{31}P NMR spectra. These results were

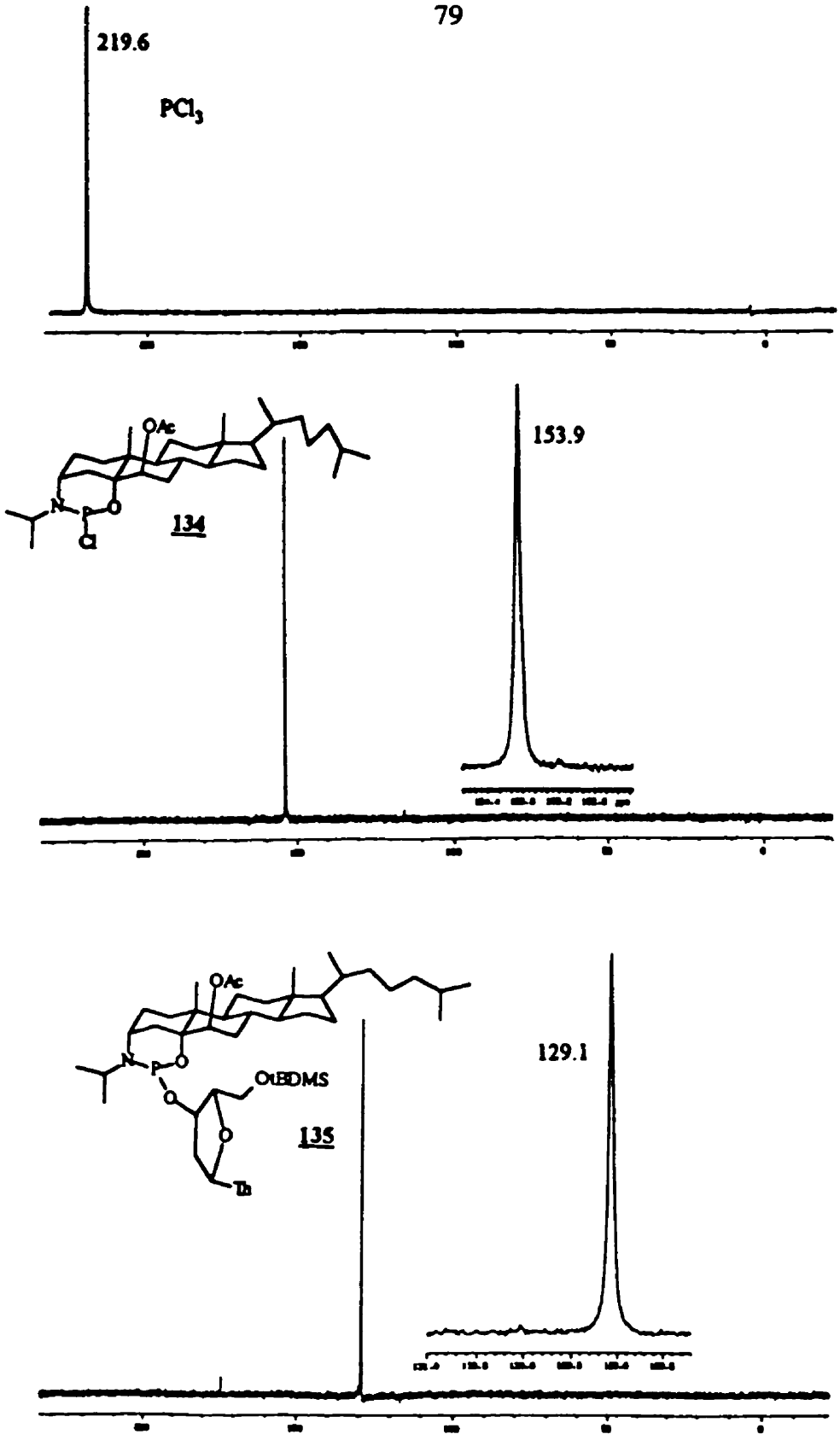


Figure 3.1: ^{31}P NMR spectra of the starting PCl_3 (top) oxazaphosphorinane 134 (center) and oxazaphosphorinane 135 (bottom)

interpreted in terms of kinetically and thermodynamically favored products. At low temperature, both products were observed, the kinetically favored product being the minor one, and the latter transformed into the thermodynamically favored one upon heating. This transformation occurred very slowly, due to a large energy barrier between the two products. When the addition was performed at higher temperature, only the thermodynamically more stable product was obtained. Oxazaphosphorinane **135** was stable to silica gel chromatography, and was characterized by means of ^{1}H, ^{13}C, ^{31}P NMR and MS. Appendixes 5 and 6 show the ^{1}H NMR spectrum and the 2D-COSY map for oxazaphosphorinane **135**. For characterization purposes, **135** was reacted with Beaucage's reagent, and cyclic thiophosphoramidate **136** could be isolated and characterized as well.

After purification of the chiral phosphoramidite derivative **135**, the displacement of the amine moiety on this group was studied. When reacted with 3 or 10 eq. tetrazole and 1 eq. 3'-O-tBDPS-thymidine at ambient temperature in acetonitrile or in chloroform, no reaction occurred even after 4h. The oxazaphosphorinane moiety was stable to these conditions, whereas an acyclic phosphoramidite derivative usually reacts under the same conditions in a few seconds, to give a phosphite triester. After 24h, about 30% of the starting compound had decomposed to several compounds having resonance signals between 0 and 15 ppm by ^{31}P NMR. However, when the catalyst was replaced by 2-bromo-4,5-dicyanoimidazole[182], the starting oxazaphosphorinane **135** disappeared within 30 min. as observed by ^{31}P NMR, yielding only hydrolysis products when the reaction was performed in acetonitrile. On the other hand, when the reaction was performed in chloroform, about 30% of a phosphite triester product could be observed after 15 min. (appearing at 141.8 ppm by ^{31}P NMR), as well as hydrolysis products having resonance frequencies between 0 and 20 ppm. After 30 min., the product was completely hydrolyzed, giving rise to several products having resonance signals between 0 and 20 ppm on the ^{31}P NMR scale.

Interestingly, the same reaction occurred when oxazaphosphorinane **135** was reacted in the presence of 2-bromo-4,5-dicyanoimidazole alone, with no 3'-tBDPS-thymidine, pointing out the acid sensitivity of the precursor.

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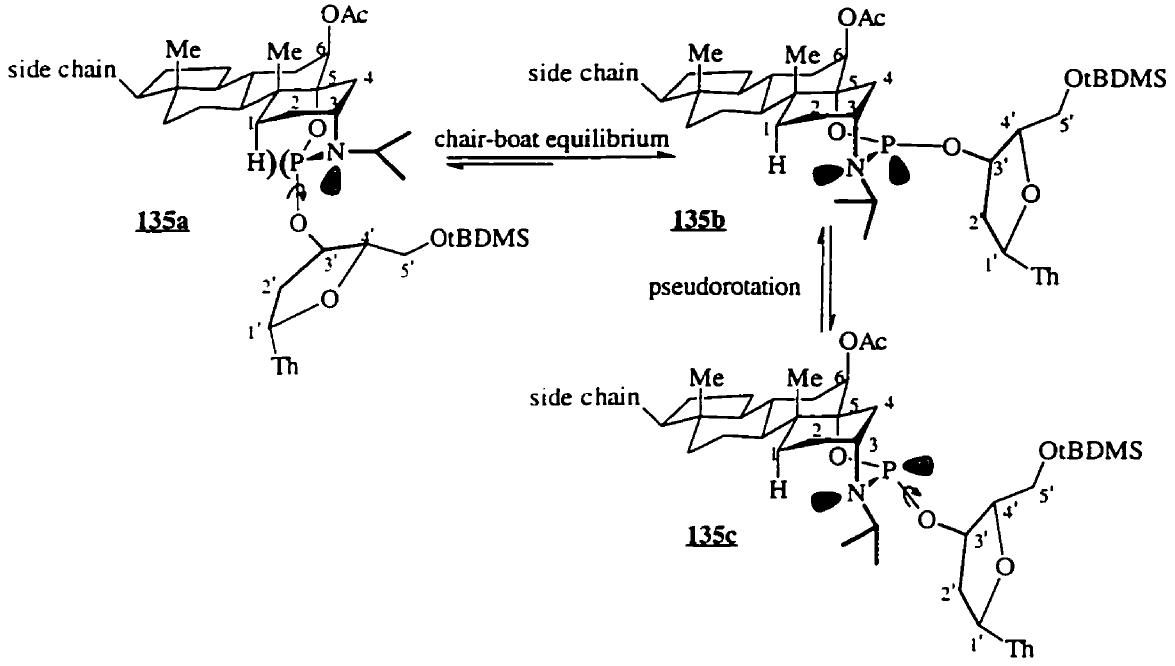
[182] Apen, P. G.; Rasmussen, P. G. *Heterocycles* **1989**, *29*, 1325

3.2.3. Structure of oxazaphosphorinane 135

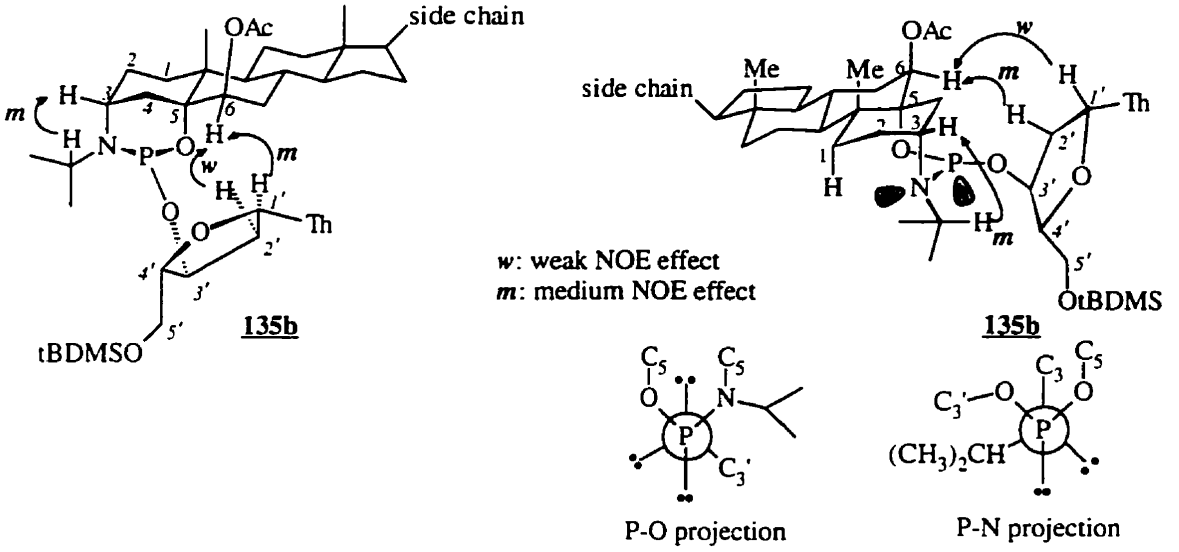
In order to rationalize these results, the possible structures for **135** were considered (scheme 3.12). The cholestane backbone imposed the conformation of the oxazaphosphorine ring to be either chair or boat. If the latter was in the chair conformation **135a**, it would bring the axial H_1 atom in very close proximity with the phosphorus atom, creating a strong steric hindrance. For this reason, it appeared impossible to have the oxazaphosphorinane ring in a chair conformation. It had to be in a boat conformation, which usually is a higher energy structure, unless steric requirements enforce it as in the present case. Therefore, the two possible structures for **135** were **135b** and **135c**,



Scheme 3.12: possible conformations of oxazaphosphorinane **135**

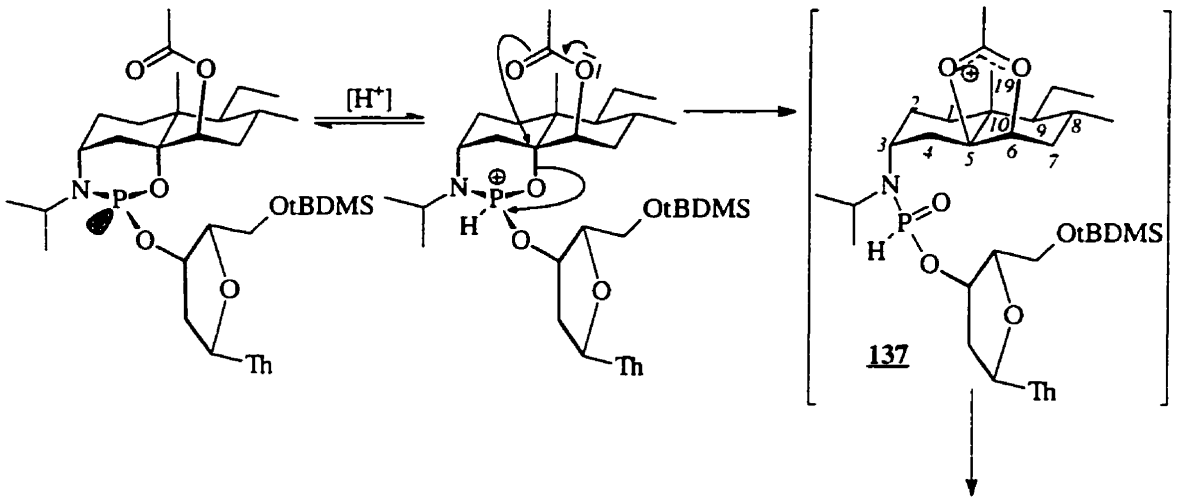
having a boat conformation with the thymidine group in axial or equatorial position respectively. The axial orientation was confirmed by the coupling constant between the phosphorus atom and the $C_{3'}$ atom of thymidine, which had a value of 20.1 Hz. Such a large coupling constant indicated a small dihedral angle between the $C_{3'}$ atom and the phosphorus lone pair, corresponding to an axial orientation of $C_{3'}$ with respect to the oxazaphosphorinane ring. Confirming this hypothesis, an NOE effect was observed between $H_{1'}$ on the thymidine ring and H_6 on the cholestane backbone, as well as between

$H_{2'\alpha}$ and H_6. Additionally, an NOE effect was observed between the isopropyl proton and H_3 on the cholestane backbone. Furthermore, the coupling constant between the isopropyl carbon atom linked to the nitrogen and the phosphorus atom was found to be $^2J_{C\text{-}P}$ = 27.5 Hz, which is in favor of structure **135b**. This structure displays a small dihedral angle between the C-N bond and the phosphorus lone pair, which is in good agreement with such a large coupling constant. Scheme 3.13 summarizes these results, appendix 7 shows the 2D-NOESY map for oxazaphosphorinane **135**.



Scheme 3.13: NOE effects in oxazaphosphorinane **135**

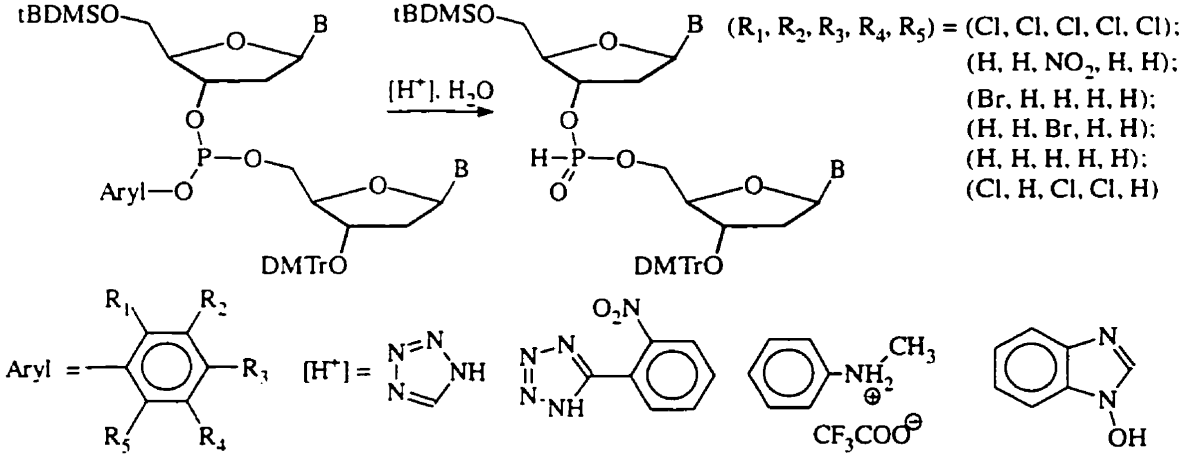
The fact that tetrazole was not able to promote any reaction was in agreement with the studies of Mrs. Yi Jin and Mr. Giancarlo Biancotto on xylose-derived precursors. Their chiral auxiliaries reacted very slowly in the presence of tetrazole. On the other hand, 2-bromo-4,5-dicyanoimidazole was able to promote some reaction, either rearrangement or coupling. The fact that mostly rearrangement was observed, giving rise to a large number of products, was interpreted in terms of the ease of formation of a carbonium ion at C_5 of the cholestane backbone. Once the oxazaphosphorinane moiety was protonated on the nitrogen, oxygen or the phosphorus atom, a good leaving group was present on that tertiary carbon, and the acetoxy group could then participate (scheme 3.14) to help its departure as possibly an H-phosphonamidite.



Scheme 3.14: the ease of elimination of the phosphorus moiety

The intermediate **137** thus formed could then evolve by migration of methyl group at position 19, creating again another tertiary carbocation at C_{10}, that could lose a proton to give an insaturation at C_1-C_{10} or at C_9-C_{10}. Intermediate **137** could also directly lose a proton and create an insaturation at C_4-C_5 or C_5-C_6.

In agreement with this hypothesis, Caruthers and co-workers[183] reported (scheme 3.15)



Scheme 3.15: Caruthers' acid-catalyzed phosphite triesters hydrolysis

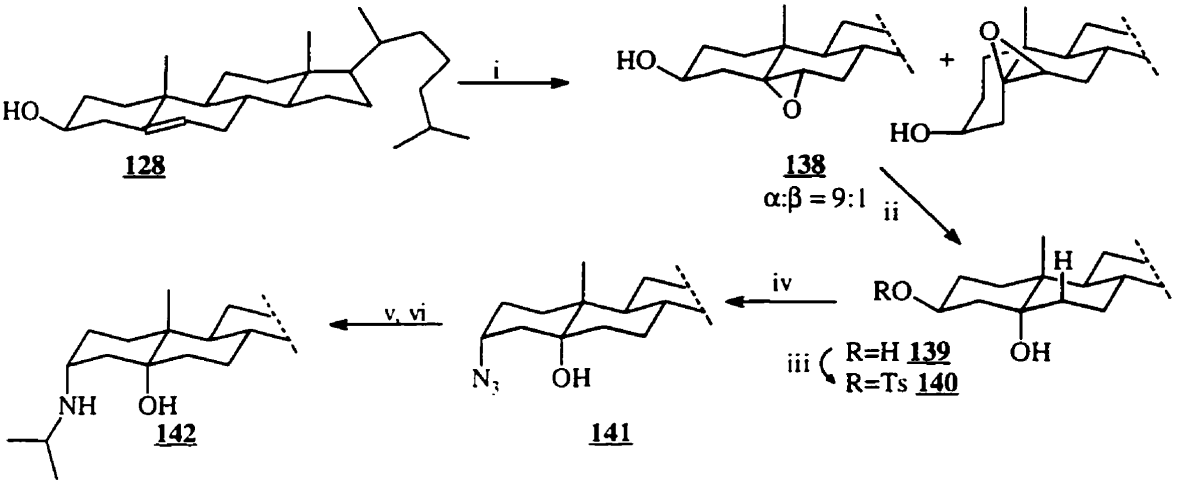
the hydrolysis of several O-aryl dinucleotide phosphite triesters, using various acids in the presence of water. They discovered that the rate of hydrolysis increased with the strength

[183] Eritja, R.; Smirnov, V.; Caruthers, M. *Tetrahedron* **1990**, *46*, 721

of the acid. Therefore it appeared necessary to reduce the ease of formation of the carbocation at C_5, and we thought of addressing this issue by synthesizing a precursor lacking the neighboring acetoxy group at C_6.

3.2.4. Use of a precursor lacking the adjacent acetoxy group

In order to determine whether the adjacent acetoxy group was the cause of the decomposition of the oxazaphosphorinane ring, we decided to synthesize a similar precursor, lacking this participating group at position 6. The synthetic route used was similar to that used for the synthesis of γ-aminoalcohol **142** (scheme 3.16).



i. mCPBA, CH_2Cl_2, 0°C then recrystallization from acetone:water; ii. $LiAlH_4$, Et_2O, Rfx;
iii. TsCl, C_5H_5N; iv. LiN_3, DMF, 100°C; v. H_2, Pd/C, EtOH;
vi. CH_3COCH_3, CH_3OH, $NaBH_3CN$, pH 5.5

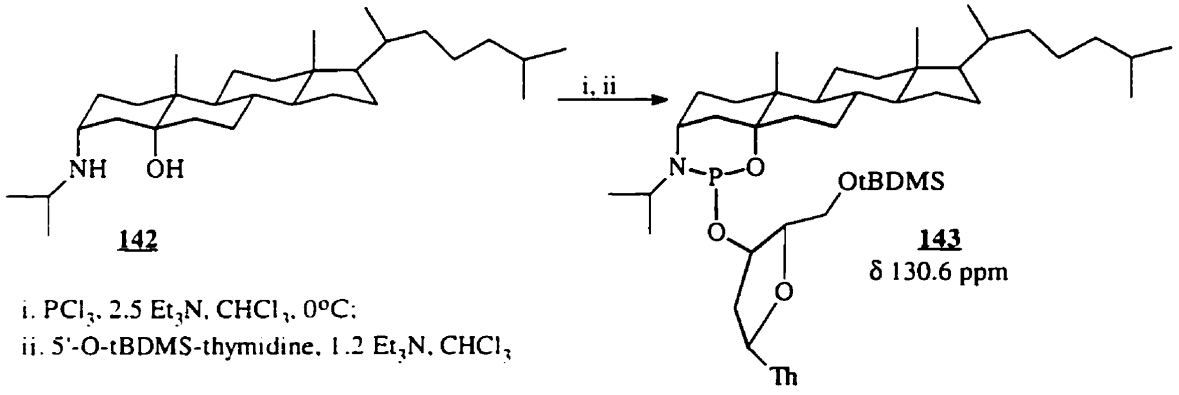
Scheme 3.16: synthesis of γ-aminoalcohol **142**

Starting from cholesterol **128**, the epoxydation of the C_5-C_6 insaturation was first performed in dichloromethane at 0°C in the presence of m-chloroperbenzoic acid (mCPBA), leading to a mixture of two diastereomers of epoxide **138** in an α:β ratio of 9:1, as evaluated from the relative integration of the ^1H NMR signals of H_3. Two recrystallizations from acetone:water led to the desired α-isomer of **138** as a pure product, m.p. 140-142°C (lit.[184] 141-143°C) in an overall 84.1% yield. Regioselective ring opening

[184] Fieser, L. F.; Fieser, M. "*Reagents for Organic Synthesis, vol. 1*" **1967**, John Wiley and Sons Eds., p. 136

of the epoxide in the presence of lithium aluminum hydride led to the desired diol **139** in 93.2% yield, m.p. 223-225°C (lit.[185] 225°C). This 3β,5-dihydroxycholest-5α-ane was then regioselectively tosylated at position 3, in the presence of p-toluenesulfonyl chloride in pyridine at 0°C, leading to the desired 3-tosylate **140** in an overall 83.1% yield after column chromatography. The tosylate was then displaced by lithium azide in dry DMF at 100°C, to yield 3α-azido-5-hydroxycholest-5α-ane **141** in 97.2% yield, m.p. 95-96°C. Catalytic hydrogenation of the latter over 10% Pd/C in ethanol led to the axial amine, which was subsequently reductively alkylated, to yield the desired N-isopropyl derivative **142**, m.p. 100-101°C in 71.2% yield starting from azide **141**.

γ-Aminoalcohol **142** was then used similarly to its analogue **125** (scheme 3.17) and was first reacted with phosphorus trichloride in the presence of 2.5 eq. triethylamine



Scheme 3.17: formation of oxazaphosphorinane **143**

in dry chloroform at 0°C. The mixture was warmed up to 50°C, then a mixture of 5'-O-tBDMS-thymidine and 1.2 eq. of triethylamine in dry chloroform was added to the reaction mixture. After 30 min. reaction, ^{31}P NMR indicated the presence of a single signal at 130.6 ppm. The desired oxazaphosphorinane **143** was purified by flash chromatography.

When it was reacted with 3'-O-tBDPS-thymidine in the presence of 2-bromo-4,5-dicyanoimidazole in chloroform, it also led to a complex mixture of products having resonance signals between 0 and 15 ppm. No reaction occurred in the presence of tetrazole.

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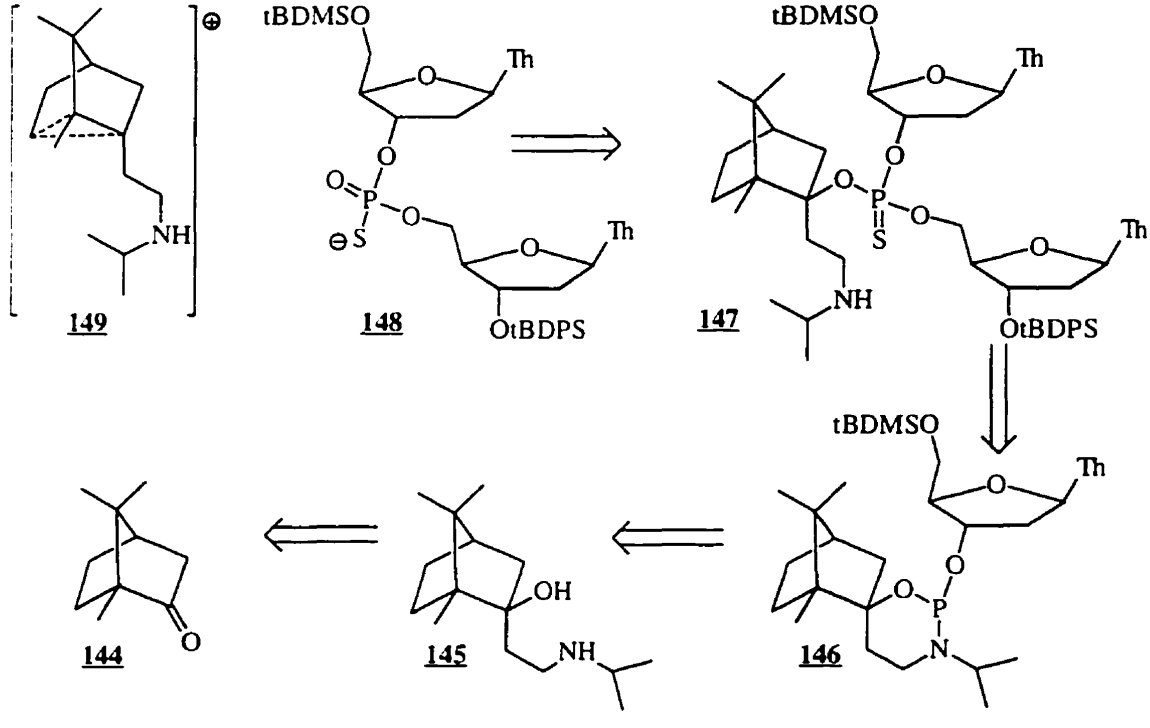
[185] Plattner, P. A.; Petrzilka, T.; Lang, W. *Helv. Chim. Acta* **1944**, *27*, 513

In conclusion, it seemed that the presence or the absence of a neighboring group at C_6 of the cholestane backbone did not make a significant difference (with respect to the present application) in the stability of the intermediate oxazaphosphorinanes **135** or **143**, or in the ease of formation of the desired phosphite triester.

The isolation of the products issued from the acid-catalyzed decomposition of oxazaphosphorinanes **135** and **143** turned out to be extremely complex, given the presence of many derivatives. ^{1}H, ^{13}C NMR and MS did not lead to any conclusive structural assignment.

3.3. Synthesis of a chiral oxazaphosphorinane derived from camphor

Terpenes are another class of natural products that also possess a built-in defined stereochemistry, and the chemistry of which is well known and allows many variations. We chose to exploit this class of compounds and make use of a chiral auxiliary derived from camphor, following the retrosynthetic strategy outlined in scheme 3.18.

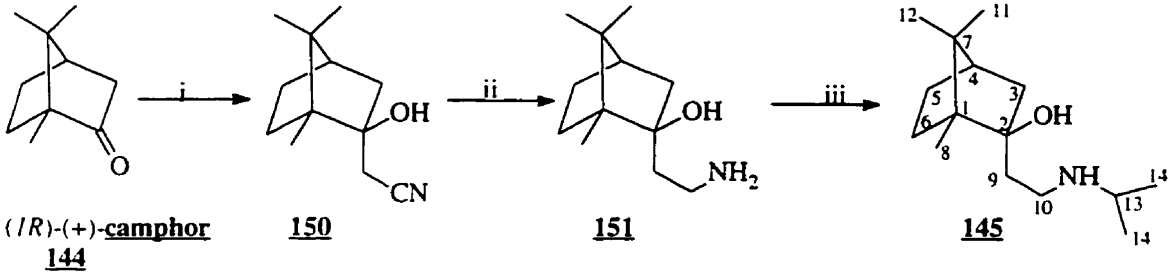


Scheme 3.18: retrosynthetic analysis of a precursor derived from camphor

Our original goal was to synthesize chiral γ-aminoalcohol **145** and transform it into chiral cyclic oxazaphosphorinane **146**. An acid-catalyzed ring opening of the heterocyclic moiety was expected to lead, after subsequent sulfurization, to phosphorothioate triester **147**. We anticipated that such a compound would be unstable enough to undergo the loss of the phosphorothioate dimer **148**, due to the ease of formation of the non-classical carbocation **149**[186]. Another advantage of this strategy was that it could make use of both enantiomers of camphor as precursors.

3.3.1. Synthesis of chiral γ-aminoalcohol 145 derived from camphor

Following this strategy (scheme 3.19), (*1R*)-(+)-camphor **144** was first reacted



(*1R*)-(+)-camphor **144** — **150** — **151** — **145**

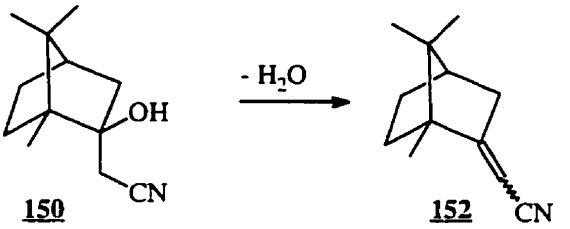
i. $LiCH_2CN$, THF, -78°C; ii. $LiAlH_4$, THF; iii. CH_3COCH_3, pH 5.5, $NaBH_3CN$, CH_3OH

Scheme 3.19: synthesis of γ-aminoalcohol **145** derived from camphor

with cyanomethyllithium in THF at -78°C. The resulting β-hydroxy nitrile **150** could not be isolated in satisfactory purity, due to its instability. This intermediate was characterized by FAB mass spectrometry and gave the expected $(M+H)^+$ pseudo-molecular ion at m/z 194, with an intensity of 7.4% with respect to the reference peak $(M-H_2O+H)^+$ at m/z 176. β-Hydroxy nitriles are known to undergo easy dehydration (scheme 3.20), which would be even easier in the present case where the alcohol is tertiary, to yield the unsaturated product **152**. Therefore, at the end of the reaction between camphor and cyanomethyllithium, the crude mixture was directly reduced with lithium aluminum hydride, with no work-up to isolate the intermediate β-hydroxy nitrile **150**. The

[186] Erman, W. F. Studies in Organic chemistry, vol.11: "*Chemistry of the monoterpenes: an encyclopedic handbook, part B*", **1985**, Gassman, P. G. Eds., pp 1078-1192

intermediate amine **151** was characterized by mass spectrometry but was not purified. It



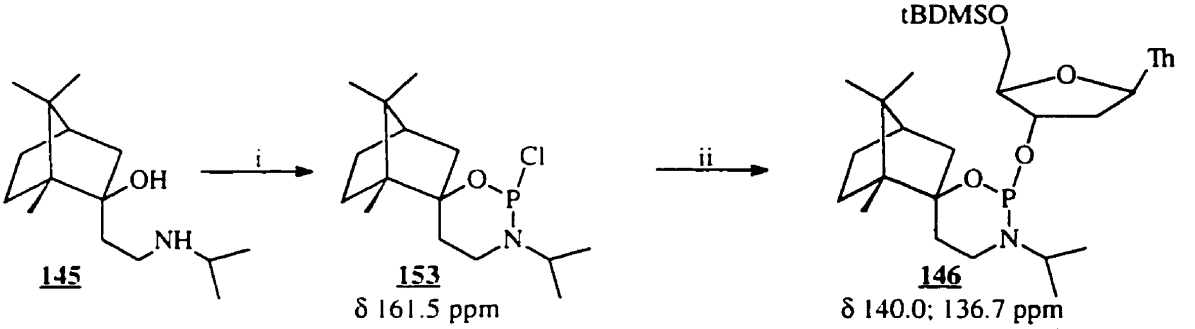
150 **152**

Scheme 3.20: elimination of water from β-hydroxynitrile

directly underwent a reductive alkylation in the presence of acetone and sodium cyanoborohydride, in methanol maintained at pH 5.5 by addition of acetic acid. The final N-isopropyl substituted amine **145** was purified by flash chromatography and could be obtained in 63.2% yield starting from (+)-camphor. Its ^{1}H NMR spectrum is shown in appendix 8, its 2D-COSY map in appendix 9.

3.3.2. Synthesis of oxazaphosphorinane 146 derived from camphor

Chiral γ-aminoalcohol **145** was then used as a chiral precursor to the formation of the corresponding oxazaphosphorinane. As indicated in scheme 3.21, the first step was



145 **153** δ 161.5 ppm **146** δ 140.0; 136.7 ppm ratio 1:1 to 3:1

i. PCl_3, 2.2 Et_3N, $CHCl_3$; ii. 5'-O-tBDMS-thymidine, 1.1 Et_3N, $CHCl_3$

Scheme 3.21: the use of **145** as a precursor

the condensation of aminoalcohol **145** with phosphorus trichloride in the presence of 2.2 eq. triethylamine or diisopropylethylamine. This reaction was carried out at 0°C in dry chloroform as a solvent, and immediately led to the formation of a single product **153** having a resonance frequency at 161.5 ppm by ^{31}P NMR. At the same temperature, a solution of 5'-O-tBDMS-thymidine and 1.1 eq. of triethylamine or diisopropylethylamine

in dry chloroform was introduced into the mixture. The ^{31}P NMR spectrum of the mixture quickly showed the presence of two oxazaphosphorinanes **146** in ratios between 1:1 and 3:1, having resonance signals at 140.0 and 136.7 ppm respectively (figure 3.2).

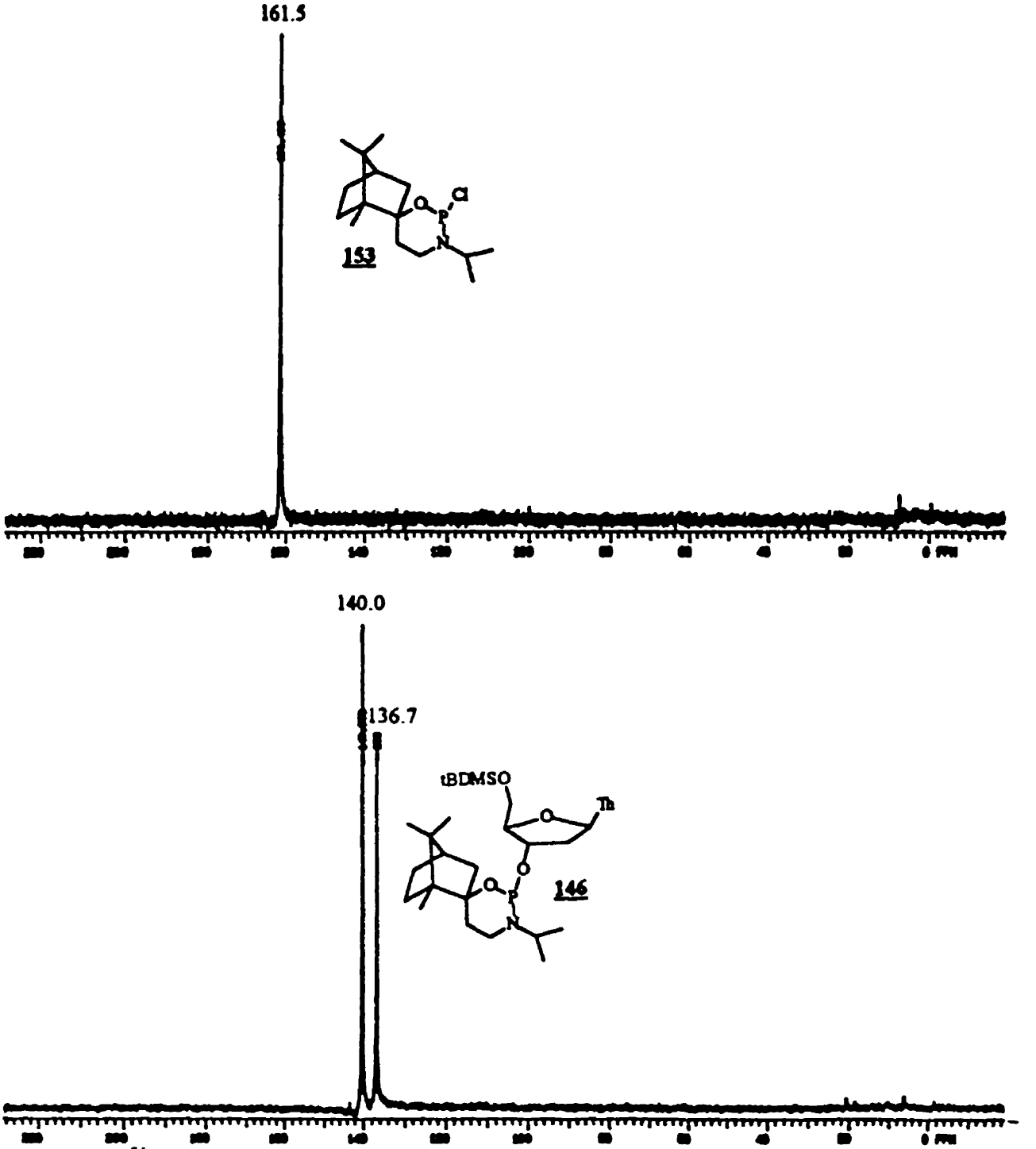
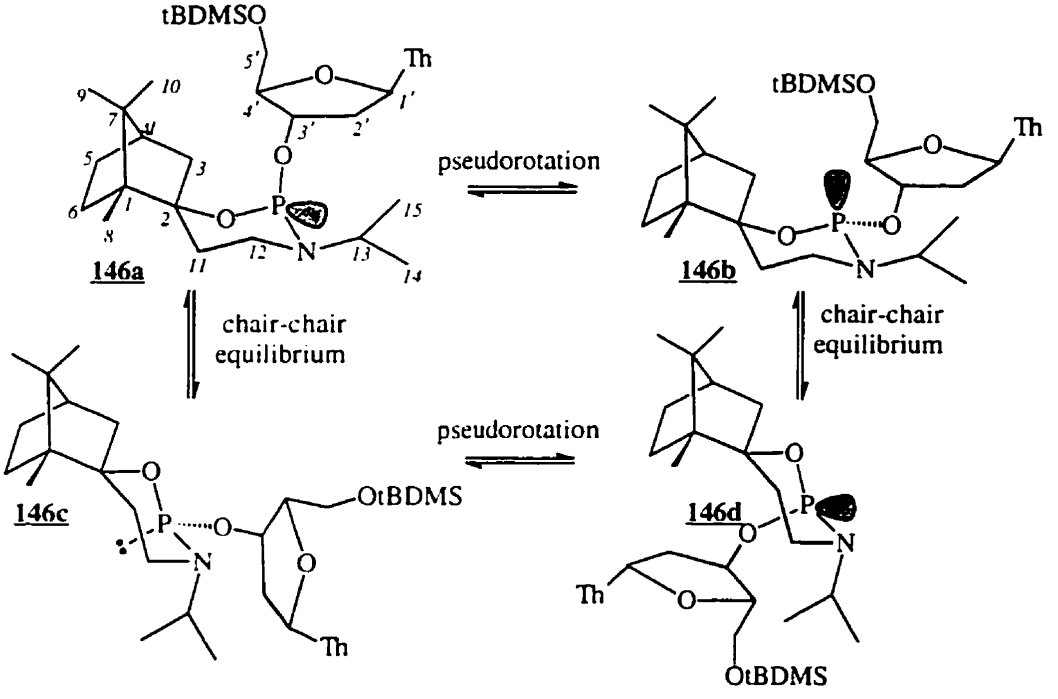


Figure 3.2: ^{31}P NMR spectra of oxazaphosphorinane **153** (top) and both diastereomers of oxazaphosphorinane **146** (bottom)

These two products were expected to convert to a single one that would be thermodynamically favored. However, upon standing at ambient temperature the composition of the mixture did not change, as evidenced by ^{31}P NMR. Therefore the solution was refluxed, but ^{31}P NMR indicated no change after 4h reflux in chloroform. After 16h reflux, both compounds were still present, as well as some decomposition products having resonance frequencies around 15 ppm. The same observation was made after 48 h reflux, the ratio had not changed but the amount of hydrolyzed products had increased to about 50%, as evaluated by ^{31}P NMR. Appendixes 10, 11 and 12 show the ^{1}H NMR, 2D-COSY and 2D-HMQC maps of oxazaphosphorinanes **146**.

We then concluded that the reaction led to the formation of two products having very close thermodynamic stability. The four simplest possible structures for oxazaphosphorinane **146** were considered as described in scheme 3.22. Forms **146a** and



Scheme 3.22: possible conformations of **146**

146b should interconvert by pseudorotation at the phosphorus center, whereas **146a** and **146c** should interconvert via a ring flipping mechanism. The situation is identical between **146c** and **146d**, which should equilibrate via a pseudo-rotation mechanism, whereas **146d** and **146b** should equilibrate via flipping of the oxazaphosphorinane ring. Xin and Just[148]

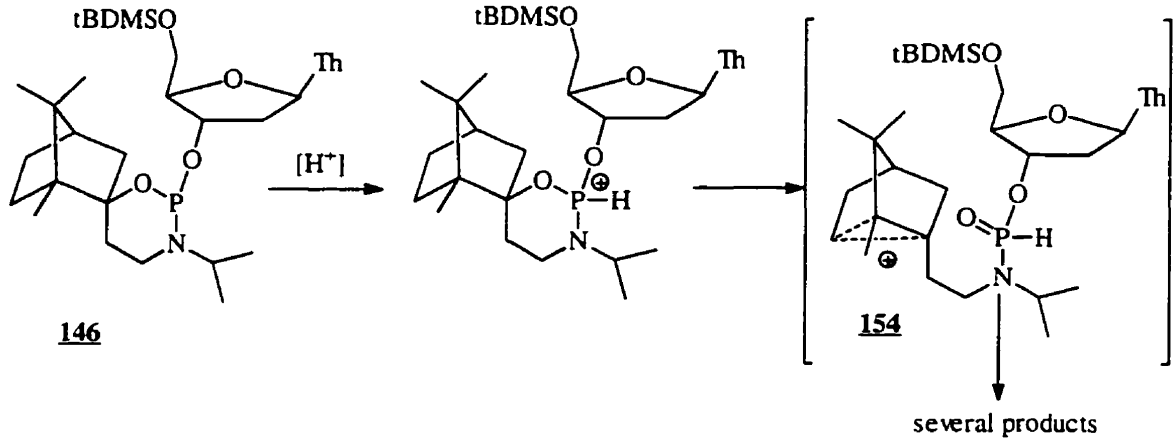
observed, during the synthesis of simple cyclic phosphoramidite derivatives, an equilibrium between the axial and equatorial forms of the oxazaphosphorinane ring **31a** and **31e** (scheme 1.16). This equilibrium evolved in favor of the thermodynamically favored axial isomer upon refluxing of the reaction mixture in the presence of triethylammonium chloride. These two observations led us to believe that the two signals observed by ^{31}P NMR corresponded to **146a** and **146d**, having both the exocyclic substituent in the axial position at the phosphorus atom. These two forms would be of equivalent thermodynamic stability, because the spiro ring junction would not create a large discrimination between the two sides of the oxazaphosphorinane ring.

Experimentally, a detailed analysis of the ^{13}C, ^{1}H, 2D-COSY and 2D-HMQC NMR spectra of a 2:1 mixture of the two diastereomers revealed that the $^{2}J_{C3'-P}$ were 21.1 and 20.1 Hz for the major and minor diastereomer respectively. This indicated that the dihedral angle between the phosphorus atom lone pair and $C_{3'}$ has to be small on both diastereomers, indicating that the exocyclic thymidine would tend to be in the axial orientation.

When this mixture of diastereomers was reacted with 3'-O-tBDPS-thymidine in the presence of tetrazole in acetonitrile, no reaction was observed initially. After 10h, some hydrolysis products were noticed between 10 and 15 ppm. When the catalyst was 2-bromo-4,5-dicyanoimidazole, a very quick reaction occurred, leading to three rearrangement products having ^{31}P NMR signals around 10 ppm. The results were the same when the oxazaphosphorinane was reacted in the presence of the catalyst alone. Similarly to the case of cholestane precursor **135**, we suspected that a good leaving group had been formed after protonation, giving rise to the non-classical carbocation **154** (scheme 3.23).

The three decomposition products could not be separated. However, the ^{1}H NMR spectrum of the mixture showed the presence of signals around 5 ppm which did not belong to the nucleoside (evidenced by COSY analysis). Additionally, HRMS (FAB) analysis of the mixture gave a pseudo-molecular ion $(M+H)^+$ corresponding to the formula $C_{31}H_{54}N_3O_6PSi + H^+$ (M+H = 624.35950), which is exactly the same as the pseudo-molecular ion corresponding to oxazaphosphorinane **146**. These two indications are

consistent with the presence of an elimination product possessing an insaturation, derived from non-classical carbocation **154**.



Scheme 3.23: hydrolysis of oxazaphosphorinane **146**

In conclusion, we discovered that γ-aminoalcohols **125** and **145** incorporating a tertiary alcohol function prone to the formation of stabilized carbocations could not be used as precursors to the diastereocontrolled synthesis of phosphite triesters. Oxazaphosphorinanes **135** and **143** derived from cholesterol could however be formed as single diastereomers, but they turned out to be very sensitive to acid-catalyzed decomposition. In addition, we discovered that the formation of oxazaphosphorinane **146** derived from camphor did not display any diastereoselectivity, and the resulting heterocyclic structure was also very sensitive to acid-catalyzed decomposition.

4. New chiral oxazaphosphorinane incorporating a participating group

4.1. The deprotection conditions

After having investigated several types of chiral γ-aminoalcohols as auxiliaries, we defined the following criteria in order to design a new chiral auxiliary.

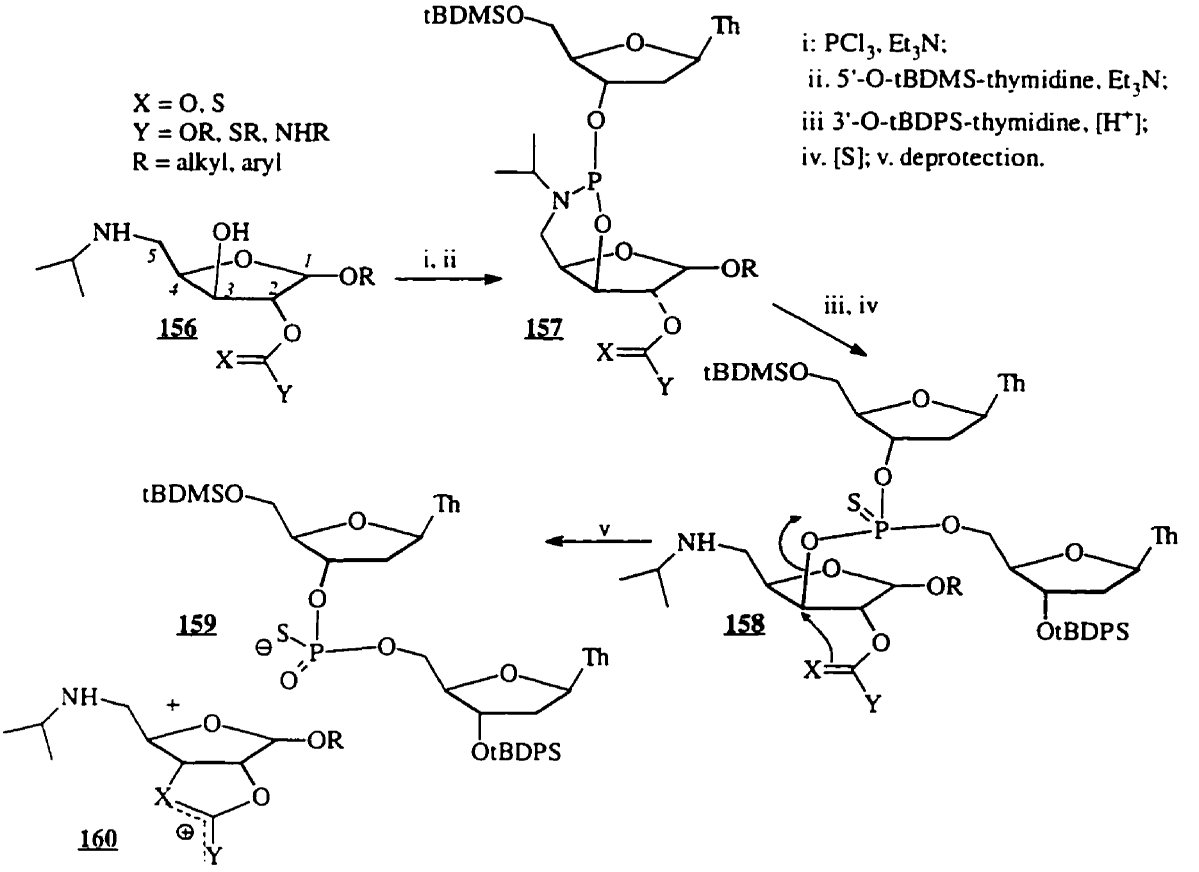
From section 2, we discovered that imidazo-oxazaphosphorines were too reactive to be used as chiral auxiliaries, since it was too difficult to introduce a nucleoside residue at the phosphorus atom. We needed, for this approach, a chiral auxiliary that would be stable enough to be stored for a sufficient amount of time and that would react very quickly when activated. It seemed reasonable to re-consider γ-aminoalcohols.

From section 3, we discovered that γ-aminoalcohols such as **125** and **145**, possessing a tertiary alcohol prone to elimination, were not suitable either because they led to oxazaphosphorinanes that would rearrange in the acidic conditions required for coupling.

The works of Mr. G. Biancotto and Mrs. Y. Jin showed that xylose could be a good precursor for a chiral auxiliary, however the deprotection conditions would have to be modified.

All these elements led us to design a new type of chiral auxiliary. This compound could be derived from a pentafuranoside structure. However, it would have to incorporate a deprotection mechanism that would be different from the 1,2-O-cyclopentylidene moiety, in order to allow the release of the phosphorothioate dimer more easily at the end of the synthesis. By analogy to the standard phosphoramidite methodology, a group that would be removable in basic conditions appeared as a suitable candidate. We therefore considered the deprotection strategy outlined in scheme 4.1. After synthesis of chiral γ-aminoalcohol **156**, we planned to prepare the corresponding chiral oxazaphosphorinane **157** bearing a thymidine residue on the phosphorus atom. This intermediate would be isolated, and the amine part of the oxazaphosphorinane ring would be subsequently displaced diastereoselectively in acidic conditions, and then sulfurized to yield phosphorothioate

triester **158**. The key step was to use a participating group to help the departure of phosphorothioate diester **159**.



Scheme 4.1: the possible use of a chiral auxiliary bearing a neighboring group

The strategy was therefore to use xylose as a support for the new chiral auxiliary, bearing an N-isopropylamino group at position 5, and a hydroxyl group at position 3. The new element was a participating group at position 2, oriented *anti* with respect to the leaving phosphorothioate moiety at position 3 of the auxiliary. Position 1 simply had to possess a well defined stereochemistry.

As indicated in scheme 4.2, participating groups have been known and studied for a few decades. Their role in substitution reactions was thoroughly studied by Winstein and co-workers[187,188,189,190], who focused initially on the acetolysis of leaving groups having a

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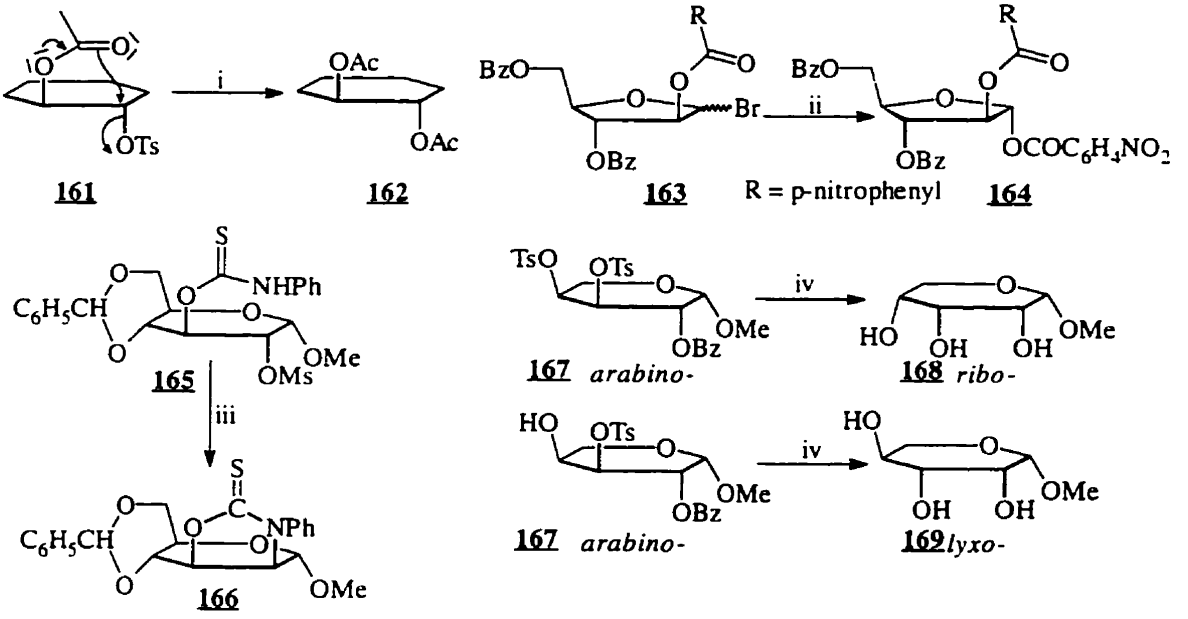
[187] Winstein, S.; Hanson, C.; Grunwald, E. *J.Am. Chem. Soc.* **1948**, *70*, 812
[188] Winstein, S.; Grunwald, E.; Buckles, R. E.; Hanson, C. *J. Am. Chem. Soc.* **1948**, *70*, 816
[189] Winstein, S.; Grunwald, E.; Ingraham, L. L. *J. Am. Chem. Soc.***1948**, 70, 821
[190] Winstein, S.; Grunwald, E. *J. Am. Chem. Soc.* **1948**, *70*, 828

neighboring acetoxy group such as **161**. In 1962, Ness[191] reported similar observations on arabinofuranose derivatives such as **163**. Later, the thiourethane[192] was cited as a good participating group as evidenced by the transformation of hexapyranoside **165** to **166**. In



i. AcOH, AcONa, 100°C; ii. pNO_2-C_6H_4COONa; iii. MeONa; iv. NaF/DMF/100 °C

Scheme 4.2: the neighboring group participation

1965, Reist *et al.*[193] described the synthesis of D-ribose and L-lyxose derivatives **168** and **169** from L-arabinose derivative **167**, with the neighboring group participation of a benzoate.

Neighboring group participation is still a commonly used way of inverting the stereochemistry of a stereogenic center with the help of an adjacent group properly functionalized. Recently, this strategy allowed organic chemists to solve some of the stereochemical problems associated with the synthesis of certain products. As indicated in scheme 4.3, Jacobsen[194] reported the synthesis of several cis β-aminoalcohols via the opening of an epoxide by a trichloroacetimido group anchored at the adjacent *anti* hydroxyl function, as indicated by the transformation from **170** to **171**. The trichloro-

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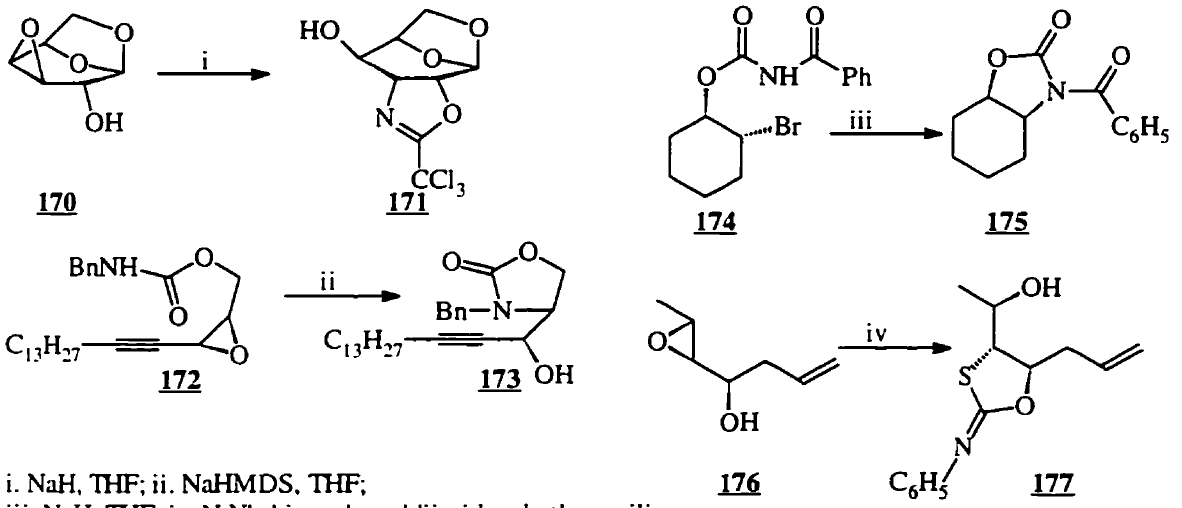
[191] Ness, R. K. *J. Org. Chem.* **1962**, *27*, 1155
[192] Baker, B. R.; Hewson, K.; Goodman, L.; Benitez, A. *J. Am. Chem. Soc.* **1958**, *80*, 6577
[193] Reist, E. J.; Fisher, L. V.; Gueffroy, D. E. *J. Org. Chem.* **1965**, *31*, 226
[194] Jacobsen, S. *Acta Chem. Scand. B* **1988**, *42*, 605

acetimido group could be removed with aqueous trifluoroacetic acid to yield the corresponding β-aminoalcohol. Bernet and Vasella[195] used a similar strategy, reacting the hydroxyl group adjacent to an epoxide with an alkyl or aryl isocyanate to yield carbamate



Scheme 4.3: recent examples of the neighboring group participation

172. This created a neighboring group that participated in the presence of a strong base (NaHMDS) to yield oxazolidinone **173**. The latter could finally be hydrolyzed to the corresponding aminoalcohol by treatment with lithium in ammonia, followed by alkaline ethanolysis. Similar participation was observed when benzoyl isocyanate[196] was used to functionalize bromohydrin **174**. N-benzoyl oxazolidinone **175** was obtained after treatment with sodium hydride. Removal of the oxazolidinone was effected more smoothly than in the previous example, with the use of aqueous sodium hydroxide. Finally, Roush and Gustin[197] recently published an elegant method, according to which α-hydroxy epoxide **176**, upon treatment with thiocarbonyldiimidazole followed by reaction with aniline, led to the formation of **177** with no need of a stronger base.

In search of a good participating group to attach to chiral auxiliary **156** (scheme 4.1), we needed a function that would have to be installed before the phosphoramidite chemistry would start. It was impossible, for example, to keep a free hydroxyl group at

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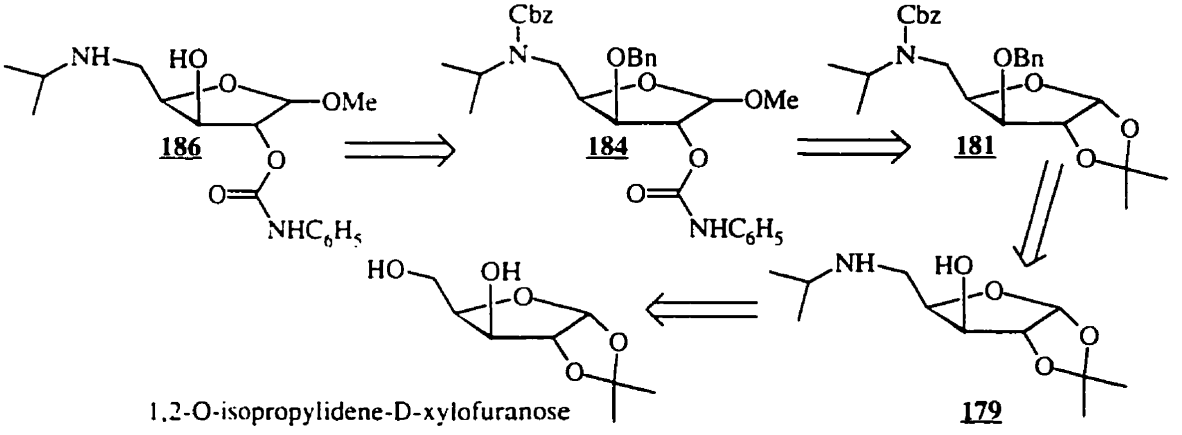
[195] Bernet, B.; Vasella, A. *Tetrahedron Lett.* **1983**, *24*, 5491
[196] Knapp, S.; Kukkola, P. J.; Sharma, S.; Pietranico, S. *Tetrahedron Lett.* **1987**, *28*, 5399
[197] Roush, W. R.; Gustin, D. *Tetrahedron Lett.* **1994**, *35*, 4931

position 2 of the xylose derivative during the reactions with phosphorus trichloride, since it would have reacted at the phosphorus atom. Therefore, the trichloroacetimido group had to be ruled out, since it had to be installed right before its participation, being a very reactive functionality.

We also wanted to differentiate hydroxyl groups 2 and 3 during the synthesis, since position 2 had to be functionalized with the participating group and position 3 had to bear a free hydroxyl. Therefore position 3 had to be protected while position 2 would be functionalized with an isocyanate. We chose to protect the hydroxyl at position 3 and the amine at position 5 with groups that would be removable in similar conditions, in order to have a single deprotection step. We therefore chose to use a benzyl group to protect the hydroxyl at position 3 and a carboxybenzyl group to protect the amine at position 5 (the benzyl group was known to be difficult to remove by hydrogenation on hindered secondary amines). Starting with a 1,2-O-isopropylidene protecting group would allow for the differentiation between hydroxyl groups at positions 2 and 3. Finally, the stereochemistry at carbon 1 was not considered as a key issue, since this position would not be involved in the phosphoramidite coupling. Scheme 4.4 presents the retrosynthetic

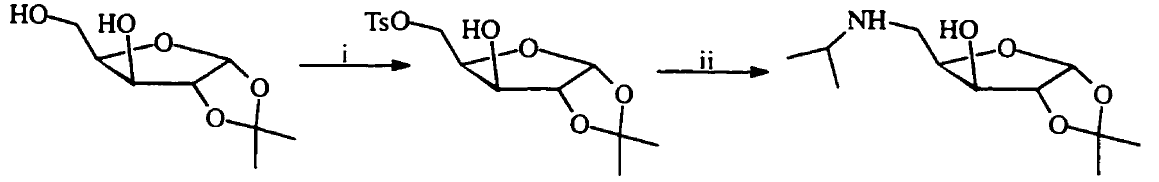


Scheme 4.4: retrosynthetic analysis of chiral auxiliary **186**

pathway planned for the synthesis of new γ-aminoalcohol **186**. It was important to introduce the participating group late in the synthesis of the chiral auxiliary, since it would then be easier to try different groups if necessary.

4.2. Synthesis of a γ-aminoalcohol incorporating a participating group

The synthesis started with commercially available 1,2-O-isopropylidene-D-xylofuranose, which was first regioselectively tosylated at position 5 in pyridine at 0°C (scheme 4.5). Tosylate **178** was then reacted with neat N-isopropylamine (b.p.^{760} 35°C)



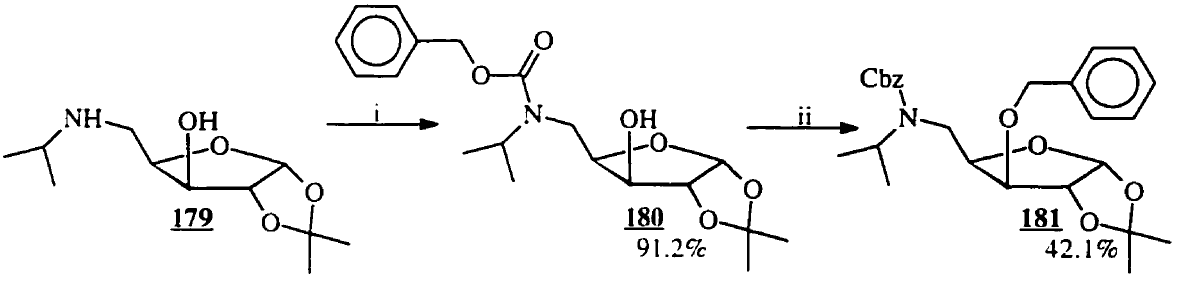
1,2-O-isopropylidene-D-xylofuranose **178** **179**

i. TsCl, C_5H_5N, 0°C; ii. neat $iPrNH_2$, 80°C, P

Scheme 4.5: functionalization of xylose at position 5

at 80°C in a pressure vessel for 16h, to yield γ-aminoalcohol **179** in a quantitative yield as an amber oil. No trace of product arising from the elimination of the tosylate was observed.

The amine was then reacted with benzyl chloroformate in a 9:1 mixture of THF

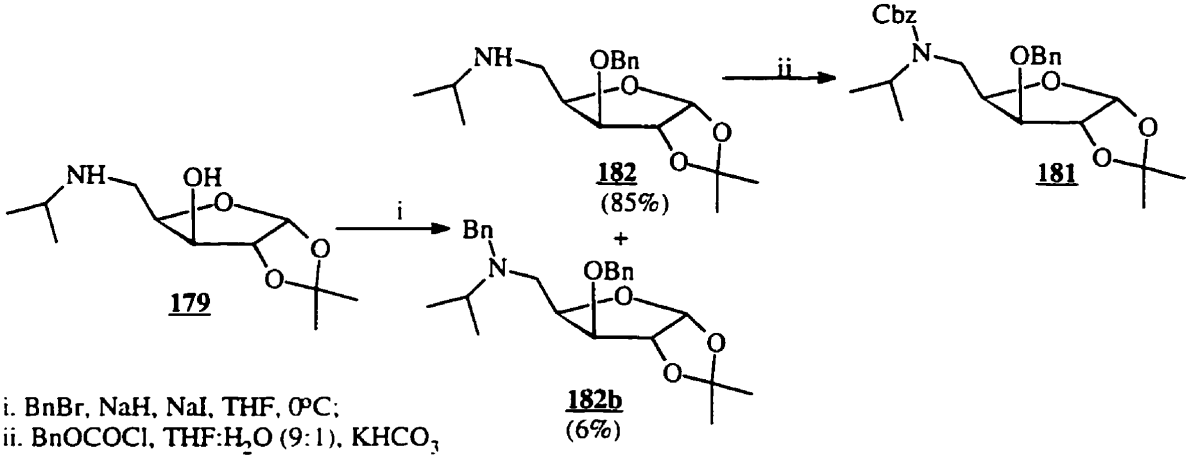


i. $C_6H_5CH_2OCOCl$, THF, H_2O, $KHCO_3$; ii. NaH, BnBr, NaI, THF, 0°C

Scheme 4.6: protection of γ-aminoalcohol **179** of positions 3 and 5

and water, in the presence of 1 eq. potassium bicarbonate at ambient temperature (scheme 4.6). After 1.5h, the N-Cbz product **180** was obtained in 91.2% yield after column chromatography, the other minor product being the O-Cbz derivative. However, upon reaction of the newly formed N-Cbz γ-aminoalcohol **180** with benzyl bromide in THF at 0°C in the presence of sodium hydride and sodium iodide, a low 42.1% yield of the desired O-benzylated product **181** was obtained, as well as other products. We concluded that the newly formed alkoxide at position 3 reacted with the N-Cbz protection at position 5.

The strategy was consequently modified, and the installation of the benzyl group was performed first, according to scheme 4.7. Upon reaction of γ-aminoalcohol **179** with



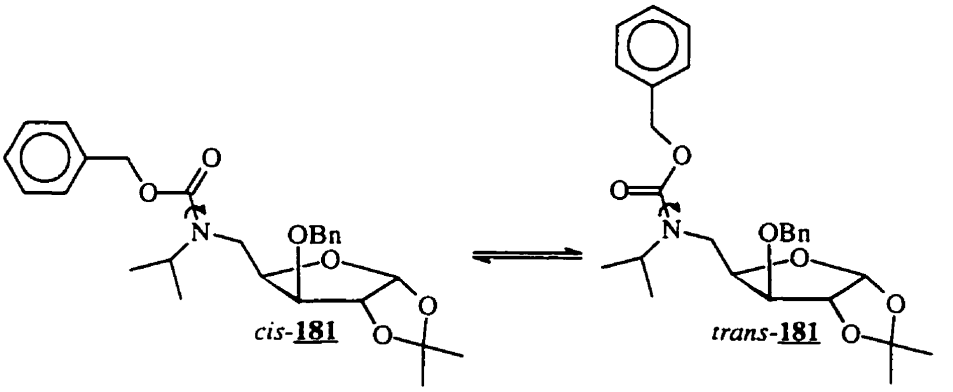
Scheme 4.7: reversal of the order of protection

benzyl bromide in the presence of sodium hydride and a catalytic amount of sodium iodide in THF at 0°C, the O-benzylated product **182** was obtained almost exclusively (85%), as well as a very small amount of the N,O-dibenzylated product **182b** (6%). Under these conditions, the sodium alkoxide seemed to have formed almost as the sole intermediate.

The amine function of O-benzylated **182** was then protected with a carboxybenzyloxy group by reaction with benzyl chloroformate in a 9:1 mixture of THF and water, in the presence of potassium bicarbonate. The reaction lasted 1.5h and gave an essentially quantitative yield of diprotected γ-aminoalcohol **181**. All the compounds synthesized after this protection of the amine by a carboxybenzyloxy group showed very broad signals by NMR (proton, carbon) when the analysis was performed at room temperature, independently of the solvent or frequency used. All the NMR analyses of compounds **181** and the following ones, containing a Cbz protection, showed the same characteristic behavior. However, when the NMR experiments were performed above 90°C, the spectra all presented sharp peaks. As indicated in scheme 4.8, this property was attributed to the well-known restricted rotation around the carbamate and amide bond[198].

[198] Dale, J. "*Stereochemistry and Conformational Analysis*" **1978**, Universitetsvorlaget, p.82

This rotation is slow at ambient temperature, at a frequency comparable to that of the



Scheme 4.8: restricted rotation around the carbamate bond

NMR experiment, constituting the cause for the broadening of the NMR signals. At higher temperature however, this rotation occurs faster as compared to the NMR time frame, translating into an averaged sharper signal.

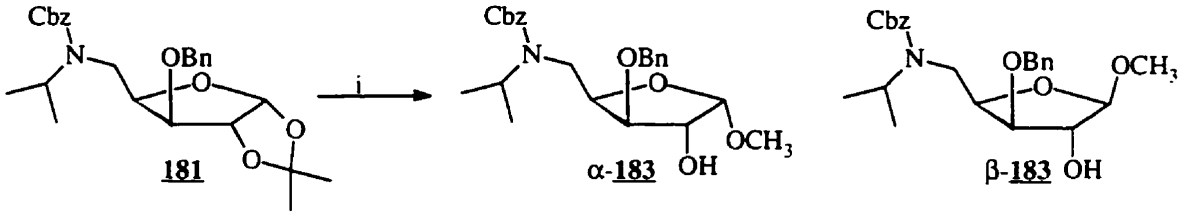
The next steps were the deprotection of the 1,2-isopropylidene group and the subsequent functionalization of position 2 with a participating group, followed by deprotection of the hydroxyl and amino functions at positions 3 and 5 respectively.

Deprotection of the 1,2-acetonide was first attempted as reported by Kawai *et al.*[199] in a mixture of acetic acid, acetic anhydride and camphorsulfonic acid or *p*-toluenesulfonic acid at 70°C. Two spots were observed by TLC, having a lower R_f than the starting compound. Separation of the two fractions revealed the presence of two compounds in each fraction, which most likely corresponded to the opened and closed forms of the sugar. Methanolysis of each mixture individually led again to a mixture of products. The acetolysis was also carried out in the presence of boron trifluoride etherate in acetic anhydride at 70°C, but led to an even more complex mixture of compounds.

Our efforts were therefore oriented towards the direct methanolysis of acetonide **181**. Reaction in a mixture of hydrogen chloride in dry methanol[200] led to the two substitution products having the methoxy group at position 1 in the α or β orientation, as well as some more polar products. A methanolysis in the presence of iodine, as reported by

[199] Kawai, S. H.; Chin, J.; Just, G. *Carbohydr. Res.* **1991**, *211*, 245
[200] Davidson, E. A. "*Carbohydrate Chemistry*" **1967**, Holt, Rinehart and Winston Eds., p. 162

Szarek *et al.*[201], yielded the best results. As indicated in scheme 4.9, two products

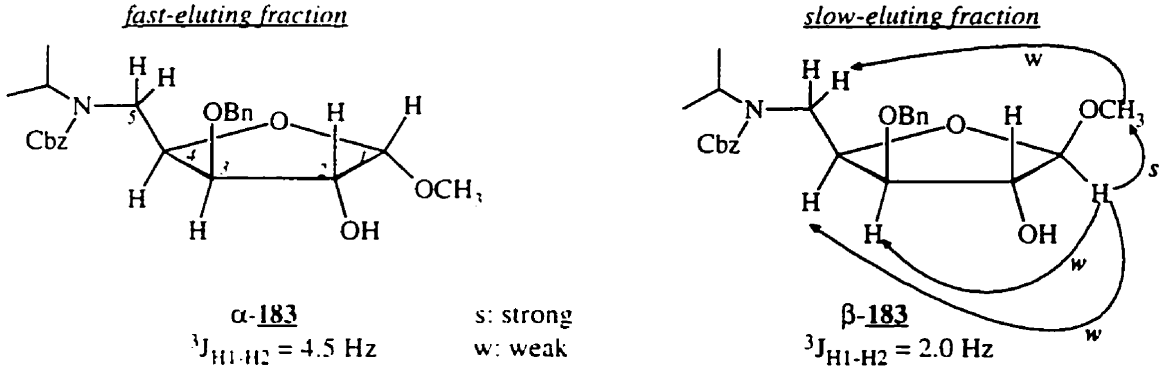


i. I_2, CH_3OH, reflux

Scheme 4.9: direct methanolysis of the isopropylidene group

were obtained after 3h reflux of acetonide **181** in dry methanol containing 1% (w/v) of iodine. The slow-eluting product was isolated after column chromatography in 53.0% yield, and the fast-eluting product in 44.0% yield.

The structural assignments for both products were deduced from the coupling constants between the protons at positions 1 and 2, and with the help of NOE experiments. Similarly to the case of their precursor **181**, NMR experiments of alcohols α-**183** and β-**183** had to be performed at 90°C in order to obtain a good resolution, owing to the restricted rotation around the N-Cbz bond. Scheme 4.10 summarizes these results.



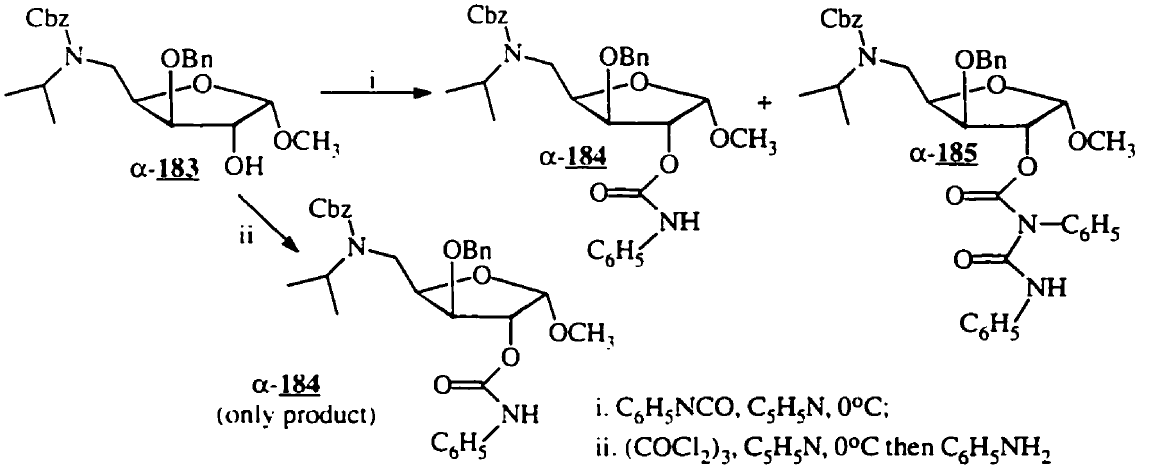
Scheme 4.10: NOE effects in the α and β anomers of **183**

The slow-eluting fraction displayed several NOE effects that were characteristic of a furanose bearing the methoxy group in the β position. On this anomer, H_1 experienced a weak NOE effect with H_3 and H_4, which could not occur if the methoxy group was in the α orientation. Similarly, the methoxy group experienced a weak NOE effect with one of the protons in position 5, which would also be inconceivable if the orientation of the

[201] Szarek, W. A.; Zamojski, A.; Tiwari, K. N.; Ison, E. R. *Tetrahedron Lett.* **1986**, *27*, 3827

former was α. The fast-eluting fraction did not display, as expected, any of these effects. According to the Karplus equation[202], the coupling constants between H_1 and H_2 of the furanose ring were also in agreement with this structural assignment. The slow-eluting fraction displayed a coupling constant of 2.0 Hz, whereas that of the fast-eluting fraction was 4.5 Hz. Even though this difference alone did not allow the rigorous assignment of the α or β orientation of the methoxy group, the larger coupling constant of 4.5 Hz for the fast eluting fraction was in agreement with a dihedral angle between C_1-H_1 and C_2-H_2 bonds that would be closer to 0°. In comparison, the smaller coupling constant of 2.0 Hz for the slow-eluting product would correspond more to a dihedral angle in the vicinity of 90°. Appendixes 13 and 14 show the difference between ^{1}H NMR spectra of alcohol α-**183** at 22°C and 90°C, appendixes 15 and 16 show the 2D-NOESY maps for alcohols α-**183** and β-**183**.

As already mentioned, the separation of α-**183** and β-**183** was relatively easy by silica gel column chromatography. The next step towards the desired γ-aminoalcohol was the functionalization of position 2 with an appropriate participating group. Our choice was oriented towards a carbamate possessing an N-H bond, so that the deprotection could be triggered by a base. Therefore, alcohols α-**183** and β-**183** were reacted separately with phenyl isocyanate in pyridine (scheme 4.11). The reaction occurred within 16h in pyridine,



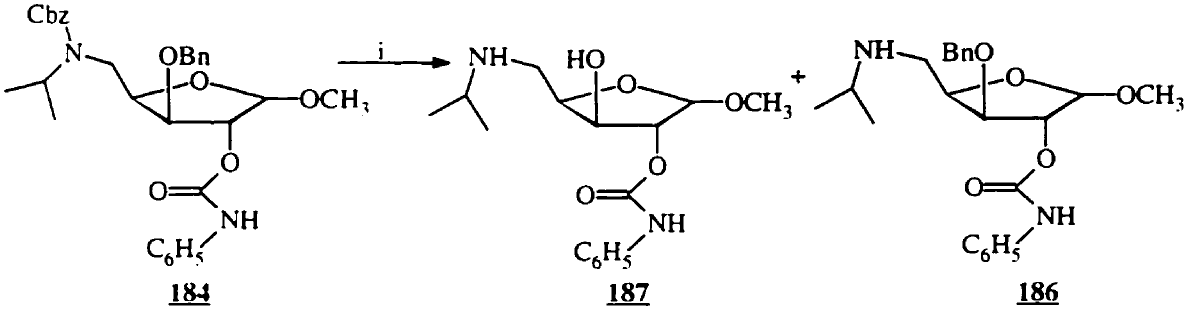
Scheme 4.11: installation of the participating group

[202] Breitmaier, E. "*Structure Elucidation by NMR in Organic Chemistry*" **1993**, John Wiley and Sons Eds. pp.42-43

starting at 0°C and continuing at ambient temperature. This procedure also led to the formation of α-**185**, a product resulting from the attack of the newly formed carbamate on phenyl isocyanate. The desired carbamate α-**184** was obtained in 71% yield, whereas the side-product α-**185** amounted to 12%, and 10% starting alcohol was recovered. However, it was difficult to separate the two compounds by chromatography, given their similar R_f. The use of methylene chloride and triethylamine instead of pyridine as a solvent did not lead to any significant improvement.

Therefore, the isocyanate was replaced by triphosgene and aniline (scheme 4.11). The starting alcohol, α-**183** or β-**183**, was reacted with one third of equivalent of triphosgene in pyridine at 0°C under argon. After 30 min., the solution turned pink, and after 1h at 0°C aniline was added. The mixture was then allowed to warm up to ambient temperature. After 12h, carbamate **184** was obtained and could be purified by flash chromatography. Both anomers gave the expected carbamate in 87 and 88% yield respectively, and the reaction could easily be scaled up to 1g.

As depicted in scheme 4.12, the last step in the synthesis of the chiral auxiliary was the deprotection of both 3-benzyl and 5-carboxybenzyloxy protective groups by



i. H_2, 10% Pd/C, C_2H_5OH:CH_3CO_2H:H_2O 8:1:1, 36 h

Scheme 4.12: removal of the Bn and Cbz protective groups

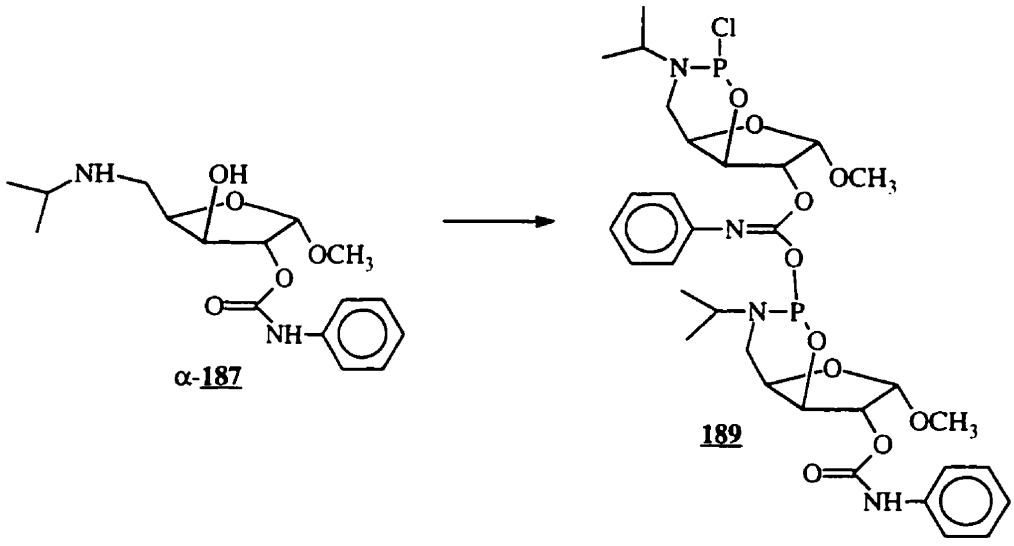
hydrogenation. The use of ethyl acetate, ethanol or methanol alone, and amounts of the catalytic agent (10% Pd/C) varying from 20% (w/w) to 100% gave a mixture of the desired aminoalcohol **187** and the O-benzyl derivative **186** resulting from incomplete deprotection of the alcohol function. This alcohol was a relatively hindered one, and conditions had to be modified in order to cleave it by hydrogenation. As shown by TLC,

the N-Cbz bond was cleaved within 3h. The hydrogenation was consequently carried out in a mixture of ethanol, acetic acid and water in a ratio of 8:1:1, and an amount of catalyst of 120% w/w with respect to protected γ-aminoalcohol **184**. The time of reaction was 36h under a pressure of 55 psi of hydrogen. Only under these conditions could both protective groups be removed completely. Both the 2-carbamate and the anomeric methoxy group survived these conditions, and the desired γ-aminoalcohol **187** could be obtained as the only product after flash chromatography, in yields of 75 to 80% for both diastereomers. NMR analyses of these compounds could be performed at ambient temperature, as a result of the loss of the carbamate function at the nitrogen atom. Appendix 17 shows the ^{1}H NMR spectrum of γ-aminoalcohol α-**187**.

4.3. Use of a chiral auxiliary possessing a participating group

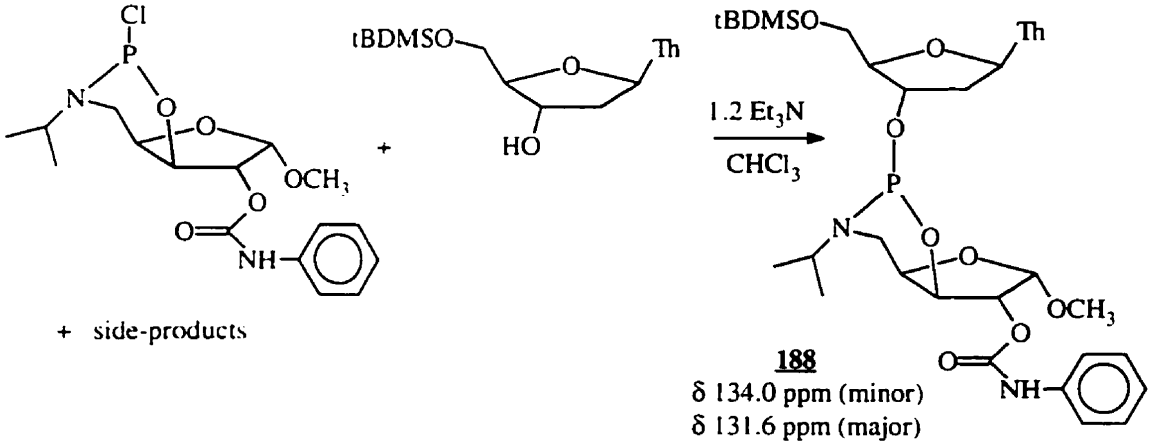
4.3.1. The synthesis of oxazaphosphorinane 188 derived from γ-aminoalcohol 187

When γ-aminoalcohol α-**187** was reacted with phosphorus trichloride in the presence of 2.5 eq. triethylamine in chloroform, a mixture of compounds initially formed. Contrary to the very fast formation of oxazaphosphorinanes derived from camphor or cholesterol, which occurred within minutes, in this case the mixture required a long time to evolve and never led to the formation of a single product. Instead, three products were formed after refluxing the mixture under argon for 48 h, showing resonance signals at 153.3, 136.4 and 129.4 ppm. These resonance signals belonged to the desired compound, as well as possibly to a product issued from the reaction of the latter with the carbamate function of the participating group such as **189** (scheme 4.13).



Scheme 4.13: possible side products during the formation of oxazaphosphorinane **188**

Nevertheless, a solution of 5'-O-tBDMS-thymidine and 1 eq. triethylamine in chloroform was injected into the reaction mixture after cooling down to 0°C. After injection, the mixture initially showed the presence of mainly two products, characterized by ^{31}P NMR frequencies at 134.0 and 131.6 ppm, attributed to both diastereomers of the desired oxazaphosphorinane **188**, in a ratio of 4:7 respectively (scheme 4.14). The



Scheme 4.14: incorporation of a 5'-O-tBDMS-thymidine residue at the phosphorus atom

mixture required refluxing for 24 h in order to equilibrate to a single diastereomer at 131.6 ppm. This single diastereomer was separated from the reaction mixture by silica gel column chromatography and was isolated in 67.3% yield as a white solid. It was then characterized by ^{1}H, ^{13}C, ^{31}P NMR and MS. Appendixes 18 and 19 show the

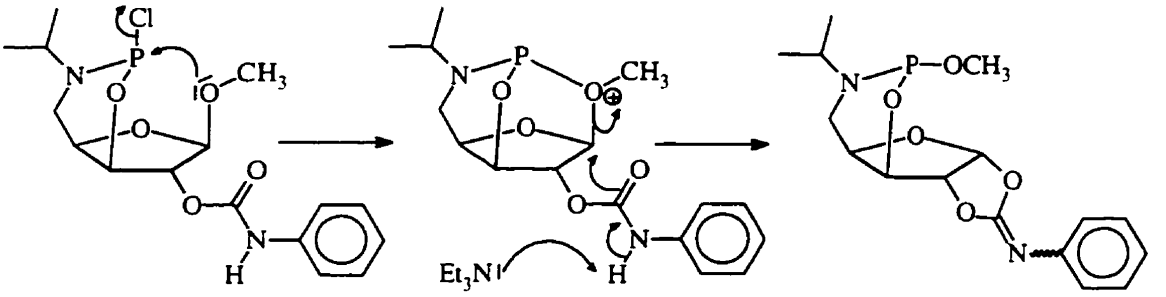
corresponding ^{1}H NMR spectrum and 2D-COSY map. The obtention of the desired product after addition of 5'-O-tBDMS-thymidine in the reaction mixture encouraged the hypothesis according to which side-products such as **189** were issued from the condensation of γ-aminoalcohol α-**187** on phosphorus trichloride. The carbamate, given its electron deficiency, is certainly a good leaving group at the trivalent phosphorus atom in the basic conditions used here. Any other group present, such as an amino or alkoxy group, would not have behaved as a leaving group under the present conditions and the obtention of **188** would have been impossible.

On the other hand, when γ-aminoalcohol β-**187**, having the opposite stereochemistry at C_1, was used as a precursor, the results turned out to be dramatically different.

This γ-aminoalcohol and 2.5 eq. triethylamine were added to a solution of phosphorus trichloride in dry chloroform at 0°C, under identical conditions as used for the α-anomer. After refluxing the mixture for as long as 72h, no further evolution was noticeable. However, ^{31}P NMR indicated the presence of a large number of compounds, having resonance frequencies between 160 and 110 ppm. A solution of 5'-O-tBDMS-thymidine and 1.2 eq. triethylamine in chloroform was subsequently injected into the reaction mixture after cooling the mixture down to 0°C. A complex mixture of compounds was obtained, which did not evolve at all upon refluxing of the mixture. After refluxing for one week under argon, the same mixture was present, giving many ^{31}P NMR signals between 150 and 110 ppm. When the solvent was replaced by dichloromethane, THF or acetonitrile, the results did not change and in each case a mixture of products was observed.

The only difference between this system and the previous one resided in the configuration of the chiral precursor at C_1. It seemed very unlikely that the carbamate group located on C_2 would directly displace the β-methoxy group on C_1, knowing that this kind of displacement normally occurs in strongly acidic conditions. The only acid present in the mixture was triethylammonium chloride, which is too weak to catalyze such reaction. One may interpret this result rather using two possible explanations. The first one would be that the orientation of the methoxy group changed the ring puckering, therefore

creating an unfavorable conformation for the oxazaphosphorinane ring to form. According to the second hypothesis, the methoxy group and the electron poor phosphorus atom would be in close proximity, allowing the P-Cl bond to play the role of a Lewis acid that could catalyze the substitution of methoxy group at C_1 by the carbamate (scheme 4.15).



Scheme 4.15: participation of the 2-carbamate at position 1

No pure compound could be isolated from this complex reaction mixture.

4.3.2. Oxazaphosphorinane 188 as a precursor to chiral TT phosphorothioate

Consequently, chirally pure oxazaphosphorinane **188** was used as a precursor in the synthesis of chirally pure phosphite triester and phosphorothioate dimer TT.

In the presence of tetrazole as a catalyst in acetonitrile, oxazaphosphorinane **188** reacted slowly, giving mostly the starting precursor as well as a mixture of phosphite triesters and hydrolysis products in the area of 10-15 ppm after 3h.

However, when the method developed by Mrs. Jin Yi was used (reaction with 3'-O-tBDPS-thymidine in the presence of 2 eq. 2-bromo-4,5-dicyanoimidazole as a catalyst and chloroform as a solvent at -5°C), the precursor started disappearing within 5 min. After 1h reaction, 10-15% of the precursor was still present, with the desired phosphite triester as a single diastereomer as indicated by its resonance signal at 140.8 ppm, as well as several H-phosphonate products appearing between 0 and 18 ppm. Either the starting oxazaphosphorinane rearranged in acidic conditions, before it had time to react with 3'-O-tBDPS-thymidine, or the product phosphite triester reacted after its formation.

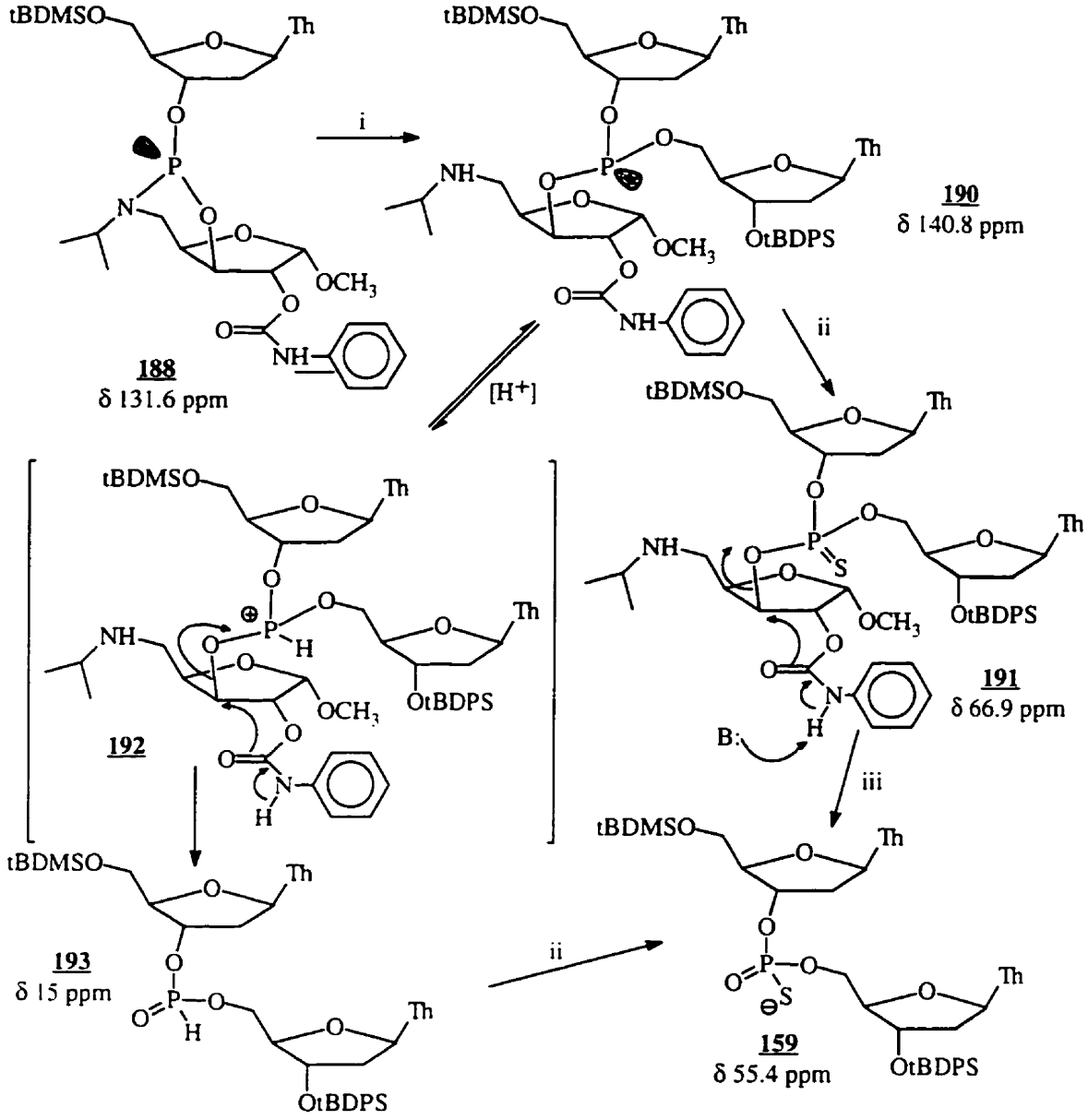
The reaction was then carried out in acetonitrile as a solvent with 4 eq. of 2-bromo-4,5-dicyanoimidazole as a catalyst, at ambient temperature. In order to prevent the decomposition of the intermediate phosphite triester to an H-phosphonate, the sulfurizing

reagent was introduced into the reaction mixture after 1 min. coupling. The ^{31}P NMR spectrum of this reaction revealed the presence of two sets of peaks. Two phosphorothioate triesters were observed at 66.9 and 65.8 ppm, in a ratio of 10:1 respectively, and two phosphorothioate diesters at 55.4 and 54.6 ppm, in a ratio of 6:1 respectively. The first set of peaks accounted for about 60% of the mixture and the second for 40%, as evaluated by the integration of the peak areas. After 24h, the ^{31}P NMR spectrum of the mixture remained unchanged, indicating that the products were stable under these acidic conditions. The introduction of 28% aqueous ammonia in the reaction mixture led, after a few minutes, to the transformation of the triesters into diesters, revealing that the adjacent carbamate group at position 2 had indeed participated and prompted the elimination of the phosphorothioate diester. The result was a pair of diastereomers of the resulting phosphorothioate diester in a ratio of 8:1.

Therefore the experiment was repeated, this time in acetonitrile at -20°C, using 4 eq. 2-bromo-4,5-dicyanoimidazole as a catalyst. After 1 min. reaction, Beaucage's sulfurizing reagent was introduced into the reaction mixture. ^{31}P NMR revealed the presence of two products, one at 66.9 ppm corresponding again to the phosphorothioate triester, the second at 55.4 ppm corresponding to the diester, in a ratio of 5:1 respectively. At that lower temperature, less elimination had taken place and the diastereoselectivity had increased. After introduction of aqueous ammonia into the reaction mixture, two products were collected at 55.4 and 55.1 ppm, in a ratio of 28.5:1, corresponding to both diastereomers of the desired phosphorothioate diester **159**.

Scheme 4.16 summarizes these observations. First, oxazaphosphorinane **188** reacted with 3'-O-tBDPS-thymidine in the presence of 2-bromo-4,5-dicyanoimidazole in a diastereoselective way to give the corresponding phosphite triester **190** (δ 140.8 ppm). A fraction of his intermediate, in the presence of the acid and the participating group, protonated and underwent an elimination to H-phosphonate **193** (δ 15 ppm). The result was a mixture of phosphite triester **190** and H-phosphonate **193**. Beaucage's reagent sulfurized both products with retention of stereochemistry. This step yielded on one hand the desired phosphorothioate triester **191** (δ 66.9 ppm), on the other hand phosphorothioate diester **159** (δ 55.4 ppm). This explains why two products were obtained

after these two steps. Base treatment allowed very quickly the elimination of



i. 3'-O-tBDPS-thymidine, 2-bromo-4,5-dicyanoimidazole, CH3CN; ii. Beaucage's reagent; iii. NH_4OH

Scheme 4.16: synthesis of T-T phosphorothioate dimer

diester **159** from triester **191**, with the help of the participating group at position 2 of the xylose precursor. Consequently, after base treatment, one product only was obtained, as a mixture of diastereomers in a ration of 28.5:1 in the best conditions. Figure 4.1 shows the corresponding ^{31}P NMR spectra, appendixes 20 and 21 the ^{1}H NMR spectrum and 2D-COSY map of dimer **191**.

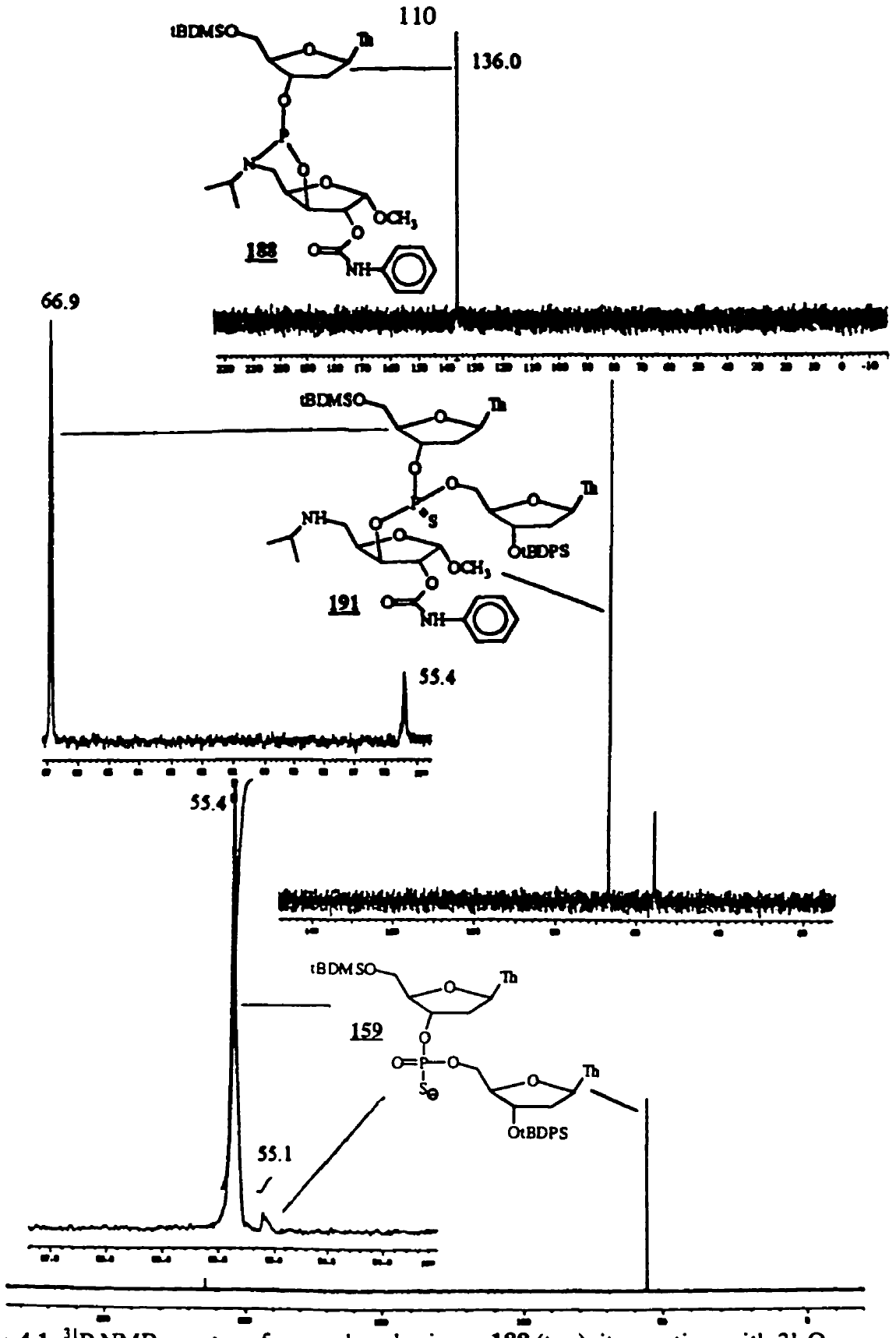
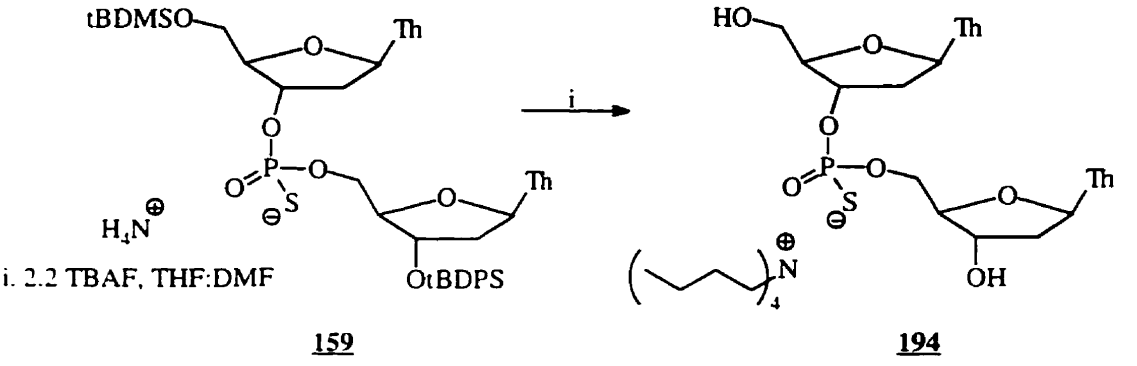


Figure 4.1: ^{31}P NMR spectra of oxazaphosphorinane **188** (top), its reaction with 3'-O-tBDPS-thymidine and sulfurization (center), and after deprotection (bottom)

It appeared from this experiment that the carbamate used here was too good a participating group, if one wanted to isolate all the intermediates. It ought to be made less electron rich by substitution with by more electron withdrawing groups on the aromatic ring. In these conditions, it would require the use of a base to participate, instead of partially participating in the absence of a base.

After purification by column chromatography, the major diastereomer of diester **159** was collected in 91% yield. It was then deprotected in the presence of 2.2 eq. of tetrabutylammonium fluoride (TBAF) in dry DMF, to yield the final fully deprotected phosphorothioate dTdT dimer **194** as the tetrabutylammonium salt (scheme 4.17). Appendixes 22, 23 and 24 show the ^{1}H NMR spectrum, 2D-COSY and 2D-HMQC maps of dimer **194**.



159 **194**

Scheme 4.17: full deprotection of the phosphorothioate dimer

The assignment of the absolute stereochemistry of the major diastereomer of this mixture was done using snake venom phosphodiesterase digestion and HPLC analysis. The major component of the mixture was digested by snake venom phosphodiesterase, indicating it possessed the (R_P) stereochemistry.

To conclude, we showed that a participating group could greatly improve the deprotection conditions. The use of a phenyl carbamate group in an *anti* orientation with respect to the newly formed phosphorothioate dimer could trigger the separation of the latter from the chiral auxiliary. This constitutes a new approach to the diastereoselective synthesis of phosphorothioates.

This approach could in principle be used in the synthesis of other types of P-chiral DNA analogues. The only difference would be to replace the sulfurization step by an oxidation step in the presence of an ^{18}O labelled oxidizing reagent (to yield diastereotopically labelled phosphodiester), a reaction with selenium (to yield a phosphoroselenoate) or borane dimethyl sulfide complex (to yield a boranophosphate).

5. Contribution to knowledge

A new class of heterobicyclic system, imidazo-oxazaphosphorine such as **56**, has been synthesized and its conformation studied.

This type of chiral precursor has led to the highly diastereoselective synthesis of several simple phosphite triesters, as well as their corresponding phosphorothioate triesters.

The investigation of the possibilities of introduction of a nucleoside on the phosphorus atom of imidazo-oxazaphosphorine **56** led to the elaboration of several new phosphoramidite derivatives and activated phosphite triesters.

Three new hindered oxazaphosphorinanes **135**, **143** and **146** were synthesized, all derived from γ-aminoalcohols possessing a tertiary alcohol function. All three of them led to rearrangements in acidic conditions, presumably due to the ease of formation of their corresponding non-classical carbocation.

Finally, a new oxazaphosphorinane **188** derived from xylose was synthesized and isolated. This chiral auxiliary possessed a participating group at the position adjacent to the phosphorothioate leaving group. This participating group has been shown to help the deprotection of the phosphorothioate dimer at the end of its synthesis. Oxazaphosphorinane **188** led to the diastereoselective synthesis of a T-T phosphorothioate dimer, in an $(R_P)/(S_P)$ diastereomeric ratio of 28.5:1.

6. Experimental section

6.1. General methods

Melting points were determined using a Gallenkamp MF-370 electrothermal apparatus and are uncorrected. Optical rotations were recorded on a Jasco DIP-140 digital polarimeter at the indicated wavelength, temperature, concentration (calculated in g/100 ml solvent) and solvent. MS were performed on a MS25RFA mass spectrometer in the direct-inlet mode. HRMS were performed on a ZAB 2F HS mass spectrometer.

^{1}H NMR spectra were recorded on a Varian XL200, Unity 500 or a Jeol Eclipse 270 spectrometer. ^{13}C NMR were recorded on a Varian XL200, XL300, Unity 500 or a Jeol Eclipse 270 spectrometers. ^{31}P NMR were recorded on a Varian XL300, Unity 500 or a Jeol Eclipse 270 spectrometer. Chemical shifts are always given in the δ scale in parts per million (ppm). The assignments of proton spectra are based on COSY experiments, the assignments of carbon spectra were determined with the help of APT, HETCOR or HMQC pulse sequences when appropriate. The ^{1}H NMR spectra were referenced with respect to the residual signals of deuterated chloroform (δ 7.24 ppm), methanol (δ 3.30 ppm), pyridine (δ 8.71 ppm), DMSO (δ 2.49 ppm). The ^{13}C NMR spectra were referenced with respect to the signals of deuterated chloroform (δ 77.00 ppm), methanol (δ 49.0 ppm), pyridine (δ 149.9 ppm), DMSO (δ 39.5 ppm). The ^{31}P NMR spectra were referenced externally with an 85% solution of phosphoric acid (δ 0.0 ppm). The multiplicities were given according to the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), h (heptet), m (multiplet), b (broad singlet).

Tetrahydrofuran and diethyl ether were freshly dried by distillation on sodium benzophenone ketyl, dichloromethane and chloroform on phosphorus pentoxide, methanol on magnesium, triethylamine, diisopropylethylamine and acetonitrile on calcium hydride, pyridine on barium oxide, benzene on sodium metal. Dry DMF was purchased from Aldrich Chemical Company Inc. in sure-seal bottles and was used with no further drying.

Thin layer chromatography was performed using Kieselgel $60F_{254}$ aluminum-backed plates (0.2 mm thickness) and visualized when appropriate by exposure under a U.V. light, by exposure to iodine vapors, by dipping into a solution of ammonium molybdate (2.5 g) and ceric sulfate (1.0 g) in 10% v/v aqueous sulfuric acid followed by heating, by dipping into a 1% (w/v) aqueous solution of ninhydrin followed by heating, by dipping into a potassium permanganate solution followed by light heating, or into a 10% solution of sodium sulfate followed by heating. Flash chromatography was performed using the method described by Still *et al.*[203], on silica gel Kieselgel 60 (Merck, 230-400 mesh).

Phosphorus trichloride was first degassed by refluxing for 2h under Ar followed by fractional distillation and was stored under argon. DBU, HMPT, HMDS, isopropanol, benzyl alcohol, phenyl isocyanate, 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 when appropriate and were used with no further purification unless specified. 3'-tBDPS-thymidine, tetrazole and Beaucage's reagent were generously given by Isis Pharmaveuticals (Carlsbad, Ca.).

Column chromatography of trivalent oxazaphosphorinane derivatives was performed using various ratios of ethyl acetate, hexanes and triethylamine, in all cases ethyl acetate was previously washed twice with 1/10 of its volume of a saturated solution of sodium bicarbonate, then was dried by shaking with magnesium sulfate.

All reactions involving trivalent phosphorus derivatives were performed using glassware that was heated overnight in an oven at about 140°C, then cooled down *in vacuo* and over phosphorus pentoxide for several hours and subsequently put under a dry argon atmosphere. The other glassware was heated in an oven for overnight, then cooled in a desiccator containing drierite or granular phosphorus pentoxide.

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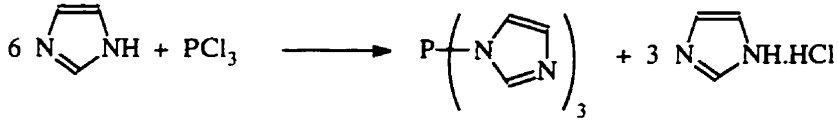
[203] Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923

6.2. Experimentals for section 2

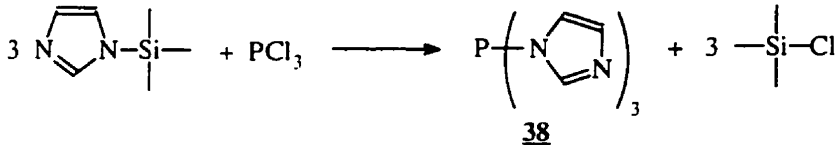
Synthesis of tri(imidazol-1-yl) phosphine 38



38

To a solution of imidazole (1.41 g, 20.7 mmol) in 24 ml dry THF stirred at 0°C under Ar was added slowly phosphorus trichloride (300 µl, 3.45 mmol). The mixture was stirred for 30 min. at 0°C, then imidazolium chloride was filtered in a schlenk tube under Ar. The turbid filtrate thus obtained was used with no further purification.

In a new procedure we developed, 1-trimethylsilylimidazole was used as a starting reagent, according to the following scheme and procedure.



38

Into a solution of 1-trimethylsilylimidazole (1.32 ml, 9.0 mmol) in 25 ml dry benzene stirred at 0°C under Ar, was syringed slowly phosphorus trichloride (262 µl, 3.0 mmol). After 30 min. stirring at 0°C, the solvent and trimethylsilyl chloride were removed *in vacuo*, taking great care not to allow any air to penetrate into the solution with the use of balloons filled with Ar. A white solid was obtained, composed of pure tri(imidazol-1-yl)phosphine 38. This solid reacted violently with air as soon as it was exposed and had to be handled under the most rigorous dry conditions.

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.70 (b, 1H, $\mathbf{H_2}$); 7.20 (b, 1H, $\mathbf{H_5}$); 7.05 (b, 1H, $\mathbf{H_4}$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 139.67 (b, $\mathbf{C_2}$); 133.48 (b, $\mathbf{C_5}$); 119.32 (b, $\mathbf{C_4}$);

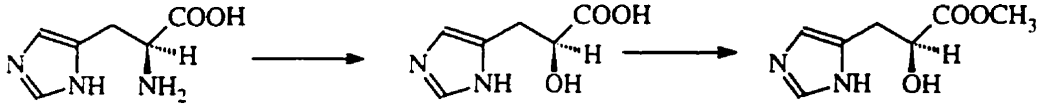
^{31}P NMR (109.4 MHz, $CDCl_3$) δ 66.6 (b);

MS (EI) m/z 232 (0.5%, $M^{+\bullet}$); 68 (100%, ($C_3H_4N_3$)).

Reaction of tri(imidazol-1-yl) phosphine 38 with isopropanol

In a glove box under an atmosphere of N_2, tri(imidazol-1-yl) phosphine **38**, obtained as a solid from our new method, was weighed (46 mg, 0.2 mmol) and introduced into a dry NMR tube. The tube was sealed with a septum, then the solvent (dry pyridine, dry acetonitrile, dry dichloromethane) was introduced, giving a turbid solution in each case. The tube was cooled down to the appropriate temperature (-78°C for dichloromethane only, -40, -20 or 0°C for the three solvents), then isopropanol (14 µl, 0.19 mmol) was syringed inside the solution. ^{31}P NMR spectra were recorded immediately at the appropriate temperature, and showed the appearance of three signals: tri(imidazol-1-yl) phosphine **38** at 66.6 ppm, di(imidazol-1-yl) isopropoxy phosphine **88** at 108.4 ppm, imidazol-1-yl diisopropoxy phosphine **89** at 125.4 ppm. Several measurements were made with variable pre-acquisition delays, but the results remained homogeneous. The ratio of products were then deduced from the relative integration of each signal.

Synthesis of methyl (*S*)-3-(imidazol-5-yl) -2-hydroxypropionate hydrochloride 48



L-(-)-**histidine** **47** **48**

L-(-)-Histidine (3.103 g, 20 mmol) was dissolved in 30 ml of 1 N aqueous hydrochloric acid solution (1.5 eq. of HCl). This solution was cooled down to 0°C, then a solution of sodium nitrite (2.070 g, 30 mmol) in 10 ml distilled water was added dropwise over a period of 1h. This yellow solution was stirred for 16h at 0°C, then evaporated to dryness *in vacuo* upon heating. Distilled water (20 ml) was added to the solid residue, and the mixture was co-evaporated twice with toluene. After drying the compound *in vacuo* for overnight and without isolation of the intermediate acid, the mixture was dissolved in 50 ml dry methanol and stirred under Ar. This solution was cooled down to 0°C and a stream of gaseous hydrogen chloride was bubbled through the mixture. After 1.5h, TLC indicated complete disappearance of the acid and the reaction was stopped. The mixture was evaporated *in vacuo* and upon heating, giving a sticky yellow solid that was

recrystallized from ethanol:diethyl ether to yield 3.10 g of **48**.HCl as a white solid, m.p. 139-142°C.

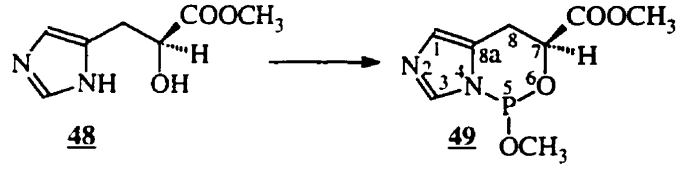
^{1}H NMR (200 MHz, CD_3OD) δ 8.90 (s, 1H, NCHN); 7.30 (s, 1H, NCHC); 4.40 (dd, X of ABX, CHOH, $^3J_{A-X}$ = 5.4 Hz, $^3J_{B-X}$ = 5.0 Hz); 3.72 (s, 3H, OCH_3); 2.85-3.10 (AB of ABX, CH_2, 2H, $^2J_{A-B}$ = 13.7 Hz, $^3J_{B-X}$ = 5.0 Hz, $^3J_{A-X}$ = 5.4 Hz,);

^{13}C NMR (assignments based on APT experiment) (125 MHz, CD_3OD) δ 175.3 (C=O); 134.2 (NCHNH); 129.7 (NCH=CNH); 117.6 (NHC=CHN); 69.9 (CHOH); 53.6 (CH3); 29.5 (CH2);

MS (CI) m/z 171 $(M+H)^+$;

$[\alpha]^D_{295}$ -21° , (c 1.9, methanol, 25°C) (lit.[204] -22°).

Synthesis of (*7S*)-7-carboxymethyl-7,8-dihydro-5-methoxy-imidazo[4,3e]oxaza-phosphorine 49



In a scrupulously dry glassware, methyl (S)-2-hydroxy-3-(imidazol-5-yl)propionate hydrochloride **48** (0.236 g, 1.39 mmol) was added and dried *in vacuo* overnight, then put under a dry Ar atmosphere. Imidazolylalcohol **48** was suspended into 5 ml dry diethyl ether and triethylamine (0.30 ml, 4.59 mmol). The suspension was cooled down to 0°C and stirred under Ar. Methyl dichlorophosphite (0.15 ml, 1.58 mmol) was then syringed into the mixture quickly. As soon as the phosphite was introduced, a thick white precipitate formed, corresponding to the production of triethylammonium chloride. After 15 min, ^{31}P NMR showed several signals between 176 and 120 ppm, as well as after 2 to 4 h. After 16h at RT, it showed a single signal at 143.5 ppm. The compound decomposed upon trying to handle it (dilution in dry ether followed by filtration in an Ar atmosphere then concentration *in vacuo*) as indicated by ^{31}P NMR by several peaks between 5 and 20 ppm. Changing the reaction conditions did not bring any improvement. Therefore,

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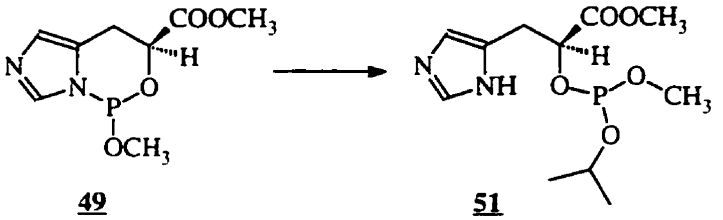
[204] Noordam, A.; Maat, C.; Beyerman, H. C. *Recl. Trav. Chim. Pays-Bas* **1981**, 441

compound **49** could not be further purified and analyzed. An attempt to sulfurize it with elemental sulfur or Beaucage's reagent resulted in a mixture of products having resonance frequencies between 0 and 55 ppm.

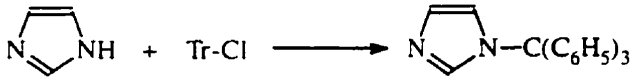
Displacement of the imidazole moiety:



49 **51**

In another experiment, imidazo-oxazaphosphorine 49 was first formed as previously described. After the observation of a single peak at 143.5 ppm, isopropanol (122 µl, 1.60 mmol) was injected into the reaction mixture. After 30 min, the conversion to a single product having a resonance signal at 140.9 ppm was observed. This signal was attributed to phosphite triester **51**. The solvents were then removed in vacuo, and the salt was precipitated by addition of 10 ml ethyl acetate (pre-washed twice with a saturated solution of sodium bicarbonate then dried over magnesium sulfate), filtered and the filtrate was evaporated *in vacuo*.. The residual was loaded on a silica gel column, and eluted with ethyl acetate:triethylamine 90:10. However, a mixture of compounds was collected and the desired product could not be isolated and characterized.

Synthesis of 1-tritylimidazole 52



1H-imidazole **52**

To a solution of trityl chloride (5.58 g, 20.0 mmol) in 100 ml dry methylene chloride cooled down to 0°C and stirred under Ar, was added dropwise over 1.5h a solution of imidazole (1.36 g, 20.0 mmol) and triethylamine (2.78 ml, 20 mmol) in 50 ml dry methylene chloride. At the end of the addition, the reaction mixture was allowed to warm up to RT and was stirred under Ar for 12h. The reaction mixture was then washed twice with 10 ml of a 10% solution of ammonium chloride, then with 10 ml of distilled water. The organic phase was dried over magnesium sulfate and evaporated *in vacuo* to

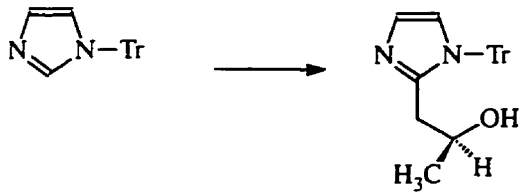
yield a white solid. Recrystallization from methylene chloride/hexanes yielded 5.60 g of a white solid, m.p. 214°C in 90% yield after recrystallization.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43 (m,1H, NC**H**N), 7.3-7.4 (m, 9H, 3xC_6**H**$_5$), 7.1-7.2 (m, 6H, 3xC_6**H**$_5$), 7.0 (m, 1H, Ph_3CNCH=CH), 6.81 (m, 1H, Ph_3CNCH=C**H**);

^{13}C NMR (50 MHz, $CDCl_3$) δ 142.3, 139.0, 129.6, 128.2, 128.3, 121.6;

MS (FAB, NBA) m/z 311 (1.8%, $(M+H)^+$); 243 (100%, $(C(C_6H_5)_3)$).

Synthesis of (*S*)-1-((1-triphenylmethyl)-imidazol-2-yl)-propan-2-ol 53



52 53

To a solution of N-tritylimidazole **52** (1.55 g, 5 mmol) in 50 ml freshly distilled THF cooled down to -78°C and stirred under dry Ar, was added a 2.5 M solution of n-butyllithium in pentane (2.4 ml, 6 mmol) over 30 min. The deep red solution thus obtained was allowed to warm up to 0°C and stirred at 0°C for 30 min, then cooled down again to -78°C. At that temperature, (S)-propylene oxide (0.35 g, 6 mmol) was added dropwise. After 30 min, the solution was allowed to warm up to 0°C and stirred at that temperature for 12h until TLC indicated that the reaction did not go further. The solution was poured into 50 ml of a 10% solution of ammonium chloride, and this mixture was extracted three times with 200 ml CH_2Cl_2. After flash chromatography (hexane:acetone:triethylamine 78:21:1), 1.44g of the desired product was collected (78 % yield) as a white solid, m.p. 201°C.

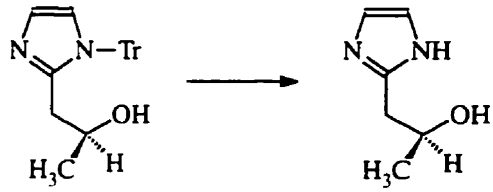
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.10 (m, 15H, 3xC_6**H**$_5$), 6.90 (d, 1H, C**H**NCPh$_3$, $^3J_{H\text{-}H}$ = 1.2 Hz), 6.71(d, 1H, C**H**=CHNCPh$_3$, $^3J_{H\text{-}H}$ = 1.2 Hz), 5.83 (b, 1H, O**H**), 3.40-3.60 (m, 1H, C**H**CH$_3$), 1.78-2.05 (AB of ABX, 2H, CH_2, $^3J_{Hb\text{-}Hx}$ = 3.2 Hz, $^3J_{Ha\text{-}Hx}$ = 8.5 Hz, $^2J_{Ha\text{-}Hb}$ = 16.2 Hz), 0.81 (d, 3H, CH_3, $^3J_{H\text{-}H}$ = 6.0Hz);

^{13}C NMR (50 MHz, $CDCl_3$) δ 149.2, 142.1, 129.6, 127.9, 127.7, 124.7, 121.0 (**N**C**C**N), 74.6, 65.0 (**C**HOH), 38.1 (**C**H$_2$), 22.3 (**C**H$_3$);

MS (CI) m/z 369 (0.3%, $(M+H)^+$); 243 (100%, $(C(C_6H_5)_3)$);

$[\alpha]^D_{295}$ -17.9° (c 0.85, $CHCl_3$).

Synthesis of (*S*)-1-(imidazol-2-yl)-propan-2-ol 54



53 54

A solution of N-tritylimidazolylpropanol **53** (2.39 g, 6.51 mmol) in 80 ml methanol containing 4.3 ml glacial acetic acid was refluxed for 12 h, until TLC indicated disappearance of the starting materials N-trityl derivative **53**. The mixture was concentrated *in vacuo* and 50 ml of cold distilled water was added to it, producing a white precipitate. The mixture was cooled down to 0°C then filtered, the white precipitate was washed with cold distilled water (10 ml). The filtrate was evaporated twice *in vacuo*, then the residual yellow oil was redissolved in 50 ml dry methanol and passed through the weakly basic anion exchange resin (hydroxide form) IRA-68. The solution was then evaporated *in vacuo* to yield a solid residue that was recrystallized from methanol:ethyl acetate to 0.76 g (93.5%) of a white solid, m.p. 119-121°C.

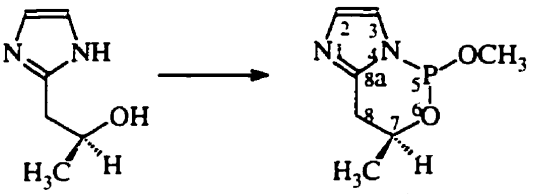
^{1}H NMR (200 MHz, CD3OD) δ 6.96 (s, 2H, NC**H**C**H**N); 3.96 (m, 1H, C**H**CH_3); 2.4-2.65 (AB of ABX, 2H, C$\mathbf{H_2}$, $^3J_{A\text{-}X}$ = 6.3 Hz, $^3J_{B\text{-}X}$ = 6.7 Hz, $^3J_{A\text{-}B}$ = 14.5 Hz,); 0.87 (d, 3H, $^3J_{H\text{-}H}$ = 6.3 Hz, C$\mathbf{H_3}$);

^{13}C NMR (50MHz, CD_3OD) δ 145.8 (N=**C**NH); 121.2 (N**C**H=**C**HNH); 67.1 (**C**HOH); 38.4 (**C**H2); 23.1 (**C**H3);

MS (CI) m/z 127 (25.3%, $(M+H)^+$);

$[\alpha]^D_{295}$ +13.4° (c 0.50, CH_3OH).

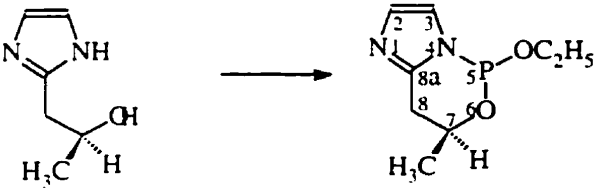
Synthesis of (*7S*) 7,8-dihydro-5-methoxy-7-methyl-imidazo[3,4-a]oxazaphosphorine 55



54 55

In an NMR tube previously dried *in vacuo* and under Ar, was introduced (*S*)-(imidazol-2-yl)-propan-2-ol **54** (23.0 mg, 0.20 mmol), then the tube was sealed with a septum and it was kept overnight *in vacuo*. After flushing with Ar, 0.7 ml dry $CDCl_3$ was introduced, followed by triethylamine (127 µl, 1.0 mmol). The alcohol did not dissolve, and this suspension was cooled down to 0°C while shaking the tube. At that temperature, methyl dichlorophosphite (18.9 µl, 0.20 mmol) was introduced into the NMR tube. Upon shaking, the alcohol dissolved instantaneously, an exothermic reaction occurred. Immediately, two products were observed at 120.6 and 118.8 ppm but after 20 to 30 min., only one was present at 118.8 ppm. The compound could not be isolated or further characterized, due to its instability.

Synthesis of (*7S*)-7,8-dihydro-5-ethoxy-7-methyl-imidazo[3,4-a]oxazaphosphorine 56



54 56

In an NMR tube previously dried *in vacuo* and under Ar, (*S*)-(imidazol-2-yl)-propan-2-ol **54** (18.9 mg, 0.15 mmol) was introduced, then the tube was sealed with a septum and the inside was put *in vacuo* for 12 h then under Ar. Dry deuterated chloroform (0.7 ml) was then introduced, followed by triethylamine (105 µl, 0.75 mmol). The alcohol did not dissolve, and this suspension was cooled down to 0°C while shaking the tube. At that temperature, ethyl dichlorophosphite (17.2 µl, 0.15 mmol) was syringed into the NMR tube. The alcohol dissolved instantaneously in an exothermic process. ^{31}P NMR

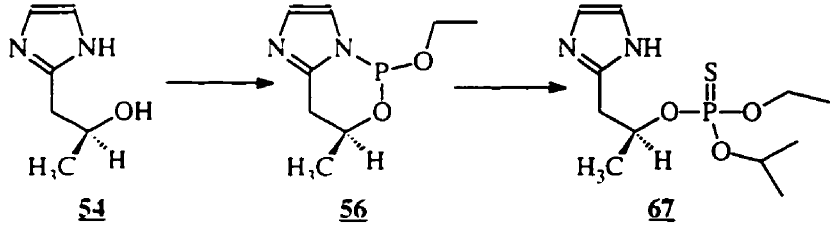
initially showed the presence of two peaks at 120.4 and 118.3 ppm, in a ratio of 1:5 to 1:8 respectively. After 20 to 30 min, the minor compound disappeared completely and one product only was observed at 118.3 ppm. The compound decomposed upon trying to handle it. A simple precipitation of triethylammonium chloride in dry ether under Ar, followed by filtration under Ar and evaporation *in vacuo*, resulted in decomposition to several products having resonance frequencies between 0 and 20 ppm. However, the compound could be used as a chiral precursor to phosphite triesters, or could be sulfurized.

^{13}C NMR (assignments based on APT experiments) (75.3 MHz, $CDCl_3$) δ 127.57 (d, C_2, $^3J_{C2\text{-}P}$ = 5.2 Hz); 115.07 (d, C_3, $^2J_{C3\text{-}P}$ = 18.1 Hz); 65.94 (d, C_7, $^2J_{C7\text{-}P}$ = 6.3 Hz); 60.23 (d, OCH_2, $^2J_{C\text{-}P}$ = 19.2 Hz); 32.74 (b, C_8, $^3JC_{8\text{-}P}$ < 0.3 Hz); 20.38 (d, $OCHCH_3$, $^3J_{C\text{-}P}$ = 4.1 Hz); 15.53 (d, OCH_2CH_3, $^3J_{C\text{-}P}$ = 5.0 Hz);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 118.3.

^1H NMR did not give any useful information due to the presence of broad signals associated with the presence of triethylamine and triethylammonium chloride that superimposed with signals arising from compound **56**.

Synthesis of ethyl (*S*)-1-(imidazol-2-yl)-prop-2-yl isopropyl phosphorothioate 67



54 **56** **67**

In a scrupulously dry NMR tube, to a suspension of (*S*)-1-(imidazol-2-yl)-propan-2-ol **54** (18.9 mg, 0.15 mmol) in 0.7 ml dry deuterated chloroform and triethylamine (0.10 ml, 0.75 mmol) shaken at 0°C under Ar was introduced ethyl dichlorophosphite (17.2 µl, 0.15 mmol). The reaction mixture was shaken at 0°C and the chiral imidazolylpropanol **54** immediately dissolved in an exothermic process. At that point, ^{31}P NMR indicated the formation of two products, one having a signal at 118.2 ppm and one having a signal at 120.3 ppm, corresponding to the two diastereomers of intermediate **56**. After 20-30 min at

RT and regular shaking of the NMR tube, ^{31}P NMR indicated only one peak at 118.3 ppm. Then, isopropanol (20 µl, 0.45 mmol) was syringed into the mixture and the tube was shaken again. After 20 min, ^{31}P NMR indicated the presence of a single peak at 140.6 ppm. So elemental sulfur (6 mg, 0.2 mmol) was introduced, which immediately produced a single compound having a resonance signal at 64.8 ppm. The product was then concentrated *in vacuo* and purified by flash chromatography (ethyl acetate/hexanes /triethylamine 79/20/1), to yield 39 mg (89.0%) of pure triester **67** as a colorless glass.

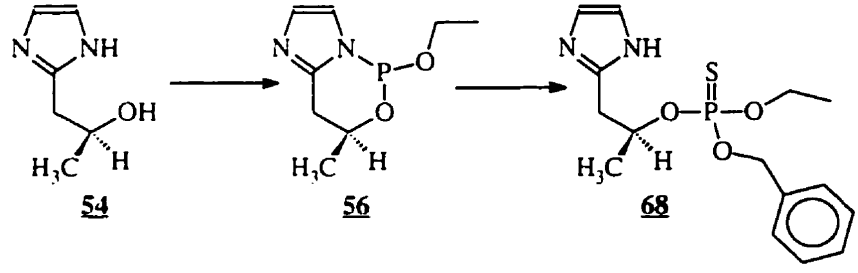
^1H NMR (200 MHz, $CDCl_3$) δ 6.96 (s, 2H, NC**H**=C**H**N); 4.83-4.97 (m, 1H, POC**H**CH_3); 4.65 (dh, 1H, OC**H**$(CH_3)_2$, $^3J_{H-H}$ = 6.2 Hz, $^3J_{H-P}$ = 9.6 Hz,); 4.05 (m, 2H, POC**H**$_2CH_3$); 2.98-3.20 (AB of ABX, 2H, C**H**$_2$CHCH_3, $^2J_{A-B}$ = 15.5 Hz, $^3J_{A-X}$ = 6.3 Hz, $^3J_{B-X}$ = 4.6 Hz, $^4J_{H-P}$ = 1.5 Hz,); 1.27 (m, 12H, CH(C**H**$_3$) + CH$(CH_3)_2$ + CH_2C**H**$_3$);

^{13}C NMR (assignments based on APT experiment) (50 MHz, $CDCl_3$) δ 144.00 (s, N**C**=N); 121.70 (s, NC=**C**N); 74.90 (d, $^2J_{C-P}$ = 6.1 Hz, POCH$(CH_3)CH_2$); 73.80 (d, $^2J_{C-P}$ = 5.7 Hz, POCH$_2CH_3$); 64.25 (d, $^2J_{C-P}$ = 5.8 Hz, POCH$(CH_3)_2$); 35.95 (s, POCH(CH_3)CH_2); 23.40 (s, POCH$(CH_3)_2$); 21.06 (s, POCH$(CH_3)CH_2$); 15.83 (s, POCH$_2$CH_3);

^{31}P NMR (81 MHz, $CDCl_3$) δ 64.8 ppm;

MS (CI) m/z 293 (100.0 %, $(M+H)^+$).

Synthesis of benzyl ethyl (*S*)-1-(imidazol-2-yl)-prop-2-yl phosphorothioate 68



54 **56** **68**

In a dry NMR tube, to a suspension of (*S*)-1-(imidazol-2-yl)-propan-2-ol **54** (30 mg, 0.24 mmol) in 1.0 ml dry deuteriochloroform and triethylamine (0.10 ml, 0.75 mmol) cooled down to 0°C under Ar, was introduced ethyl dichlorophosphite (27.5 µl, 0.24 mmol). The tube was shaken at 0°C and after 30 min, ^{31}P NMR indicated the presence of the intermediate imidazo-oxazaphosphorine **56** at 118.3 ppm. Benzyl alcohol (26.0 µl,

0.25 mmol) was then introduced. Within 5 min., ^{31}P NMR indicated the presence of a single signal at 139.8 ppm, corresponding to the intermediate phosphite triester, as a single diastereomer. Elemental sulfur (8 mg, 0.25 mmol) was introduced into the tube, and within 5 min. the previous signal disappeared, giving rise to a single signal at 66.3 ppm. The solvent was evaporated *in vacuo* and the product purified by flash chromatography, using ethyl acetate:hexanes:triethylamine 79:20:1 as an eluent. The desired phosphorothioate triester was obtained as a colorless oil in 83.5% yield (68 mg).

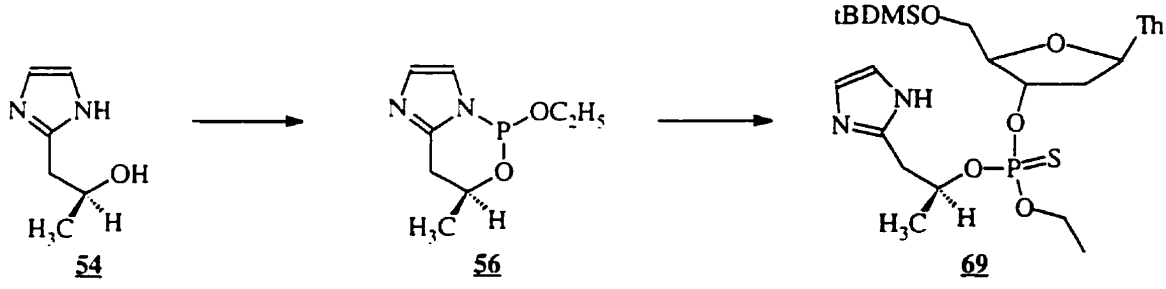
^1H NMR (200 MHz, $CDCl_3$) δ 7.35 (s, 5H, $C_6\mathbf{H}_5$); 6.81 (s, 2H, NC**H**=C**H**N); 5.00-4.80 (m, 3H, POC**H**(CH_3)CH_2, POC$\mathbf{H}_2C_6H_5$); 4.03 (m, 2H, POC$\mathbf{H}_2CH_3$); 3.00 (m, 2H, $\mathbf{CH}_2$CH(CH_3)OP); 1.29-1.20 (m, 6H, POCH$_2\mathbf{CH}_3$, POCH(C$\mathbf{H}_3$)CH_2);

^{13}C NMR δ 143.56 (N**C**=N); 137.52, 131.23, 130.95, 129.28 (aromatic **C**); 121.35 (NC=**C**N); 81.87 (d, POC$\mathbf{H}_2C_6H_5$, $^2J_{C\text{-}P}$ = 6.2 Hz); 75.36 (d, POC**H**(CH_3)CH_2, $^2J_{C\text{-}P}$ = 5.8 Hz); 74.10 (d, POC$\mathbf{H}_2CH_3$, $^2J_{C\text{-}P}$ = 6.0 Hz); 36.27 (POCH(CH_3)$\mathbf{CH}_2$); 20.87 (POCH(C$\mathbf{H}_3$)CH_2); 15.63 (POC$\mathbf{H}_2$C$\mathbf{H}_3$);

^{31}P NMR (81 MHz, $CDCl_3$) δ 66.3;

MS (CI) m/z 341 (100.0%, $(M+H)^+$).

Synthesis of (5'-*ter*butyldimethylsilyl)thymid-3'-yl ethyl (2*S*)-1-(imidazol-2-yl)prop-2-yl phosphorothioate 69



54 **56** **69**

To a suspension of (S)-1-(imidazol-2-yl)-propan-2-ol **54** (38 mg, 0.30 mmol) and dry triethylamine (0.21 ml, 1.5 mmol) in 2 ml dry chloroform cooled down to 0°C under Ar and stirring, was added ethyl dichlorophosphite (35 µl, 0.30 mmol). An exothermic reaction immediately took place. After 30 min., ^{31}P NMR indicated the presence of a single signal corresponding to **56** at 118.3 ppm. The mixture was cooled down to 0°C again, then

a solution of 5'-O-tBDMS-thymidine (107 mg, 0.30 mmol) in 1 ml dry chloroform was introduced. After 30 min, ^{31}P NMR indicated the presence of a single peak at 141.2 ppm. Elemental sulfur (11.5 mg, 0.36 mmol) was added directly to the solution, and ^{31}P NMR immediately indicated the presence of a single resonance peak at 66.3 ppm. The solvent was evaporated *in vacuo* and the mixture was purified by flash chromatography (ethyl acetate:triethylamine 90:10) to yield 127 mg (72% yield) of triester **69** as a sticky solid.

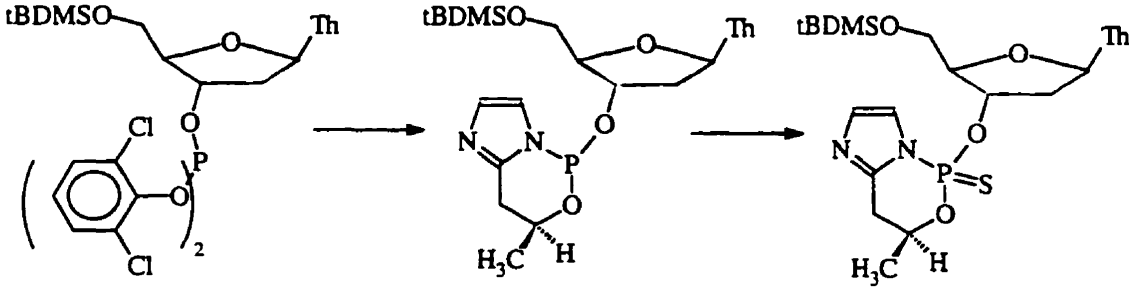
^{1}H NMR (200 MHz, CD_2Cl_2) δ 9.80 (b, 2H, 2xN**H**); 7.48 (d, 1H, CH_3C=C**H**, $^4J_{H-H}$ = 1.3 Hz); 6.94 (s, 2H, NC**H**=C**H**N); 6.25 (dd, 1H, **H$_{1'}$**, $^3J_{1'-2'a}$ = 9.2 Hz, $^3J_{1'-2'b}$ = 5.2 Hz); 4.92 (m, 2H, POC**H**(CH_3)CH_2 + **H$_{3'}$**); 4.19 (m, **H$_{4'}$**); 4.05 (dq, 2H, POC**H$_2$**CH_3, $^3J_{H-P}$ = 9.4 Hz, $^3J_{H-H}$ = 7.1 Hz); 3.84 (AB of ABX, 2H, **H$_{5'a}$**, **H$_{5'b}$**, $^2J_{5'a-5'b}$ = 11.5 Hz, $^3J_{5'b-4'}$ = 2.5 Hz, $^3J_{5'a-4'}$ = 2.4 Hz); 3.03 (dd, 2H, POCH(CH_3)C**H$_2$**, $^3J_{H-H}$ = 5.8 Hz, $^4J_{H-P}$ = 1.1 Hz); 2.33 (B of ABX, 1H, **H$_{2'b}$**, $^2J_{2'b-2'a}$ = 13.3 Hz, $^3J_{2'b-1'}$ = 5.2 Hz); 2.03 (m, 1H, **H$_{2'a}$**); 1.88 (d, 3H, CH=CH(C**H$_3$**), $^4J_{H-H}$ = 1.3 Hz); 1.39 (d, 3H, POCH(C**H$_3$**)CH_2, $^3J_{H-H}$ = 6.2 Hz); 1.3 (dt, 3H, POCH$_2$C**H$_3$**, $^3J_{H-H}$ = 7.1 Hz, $^4J_{H-P}$ = 0.9 Hz); 0.91 (s, 9H, SiC(C**H$_3$**)$_3$); 0.12 (s, 6H, Si(C**H$_3$**)$_2$);

^{13}C NMR (75.3 MHz, CD_2Cl_2) δ 164.10 (**C$_4$**); 151.07 (**C$_2$**); 144.18 (N**C**=N); 144.15 (NCH=**C**HN); 135.33 (**C$_6$**); 111.54 (**C$_5$**); 86.15 (d, **C$_{4'}$**, $^3J_{C4'-P}$ = 6.6 Hz); 85.24 (**C$_{1'}$**); 79.93 (d, POCH(CH_3), $^2J_{C-P}$ = 4.3 Hz); 76.34 (d, POCH$_2$$CH_3$, $^2J_{C-P}$ = 5.7 Hz); 65.02 (d, **C$_{3'}$**, $^2J_{C3'-P}$ = 5.6 Hz); 63.82 (**C$_{5'}$**); 39.39 (d, **C$_{2'}$**, $^3J_{C2'-P}$ = 4.3 Hz); 36.58 (d, POCH(CH_3)CH_2, $^3J_{C-P}$ = 7.5 Hz); 26.09 (SiC(**C**H$_3$)$_3$); 21.32 (POCH(**C**H$_3$)CH_2); 18.60 (Si**C**(CH$_3$)$_3$); 16.05 (d, POCH$_2$**C**H$_3$, $^3J_{C-P}$ = 6.6 Hz); 12.66 (C=**C**CH$_3$); -5.40, -5.50 (Si(**C**H$_3$)$_2$);

^{31}P NMR (121.0 MHz, CD_2Cl_2) δ 66.3;

MS (FAB, NBA) m/z 589 (100.0%, $(M+H)^+$); 339 (6.2%, (thymidine+1-H_2O)); 251 (62.2%, (ImCH_2CH(CH_3)OP(S)(OEt)OH+1)).

Synthesis of (*7S*)-5-(5'-*ter*butyldimethylsilylthymid-3'-yl)-7,8-dihydro-7-methyl-5-thio-imidazo[3,4-a]oxazaphosphorine 71b



110 71 71b

In a dry NMR tube, was introduced the activated phosphite **110** (71 mg, 0.1 mmol) and imidazolylpropanol **54** (13 mg, 0.1 mmol). The tube was kept *in vacuo* overnight, then dry THF (0.5 ml) was introduced to solubilize the activated ester, followed by DBU (33 µl, 0.22 mmol) and the ^{31}P NMR spectrum was recorded. After 30 min., it showed no further evolution and three sets of peaks were observed: the starting activated ester at 131.3 ppm, two peaks at 122.5 and 121.9 ppm in a ratio of 1:12, corresponding to imidazo-oxazaphosphorine **71**, as well as a triester at 140.0 ppm, corresponding most likely to **112**, product of the opening of the oxazaphosphorine moiety of **71** by imidazolylpropanol **54**. The ratio of **71**:**112** was 1.2:1. To this mixture was added Beaucage's sulfurizing reagent (22 mg, 0.11 mmol). After 20 min., the signals of **71** turned into two signals at 53.0 and 53.3 ppm, in a ratio of 12:1. The solvent was removed *in vacuo*, and the products were separated chromatographically, using ethyl acetate:triethylamine 85:15 as an eluent (ethyl acetate was pre-washed twice with a saturated solution of sodium bicarbonate and dried over magnesium sulfate). Only the major diastereomer of **71** could be obtained pure in a low yield (25%, 13 mg), as a colorless oil.

^1H NMR (270 MHz, CDCl3) δ 8.16 (b, 1h, C=ONHC=O); 7.47 (b, 1H, CH=C(CH_3); 7.12, 7.06 (b, 2h, NCH=CHN); 6.28 (dd, 1H, $\mathbf{H_{1'}}$, $^3J_{1'\text{-}2'}$ = 4.9 Hz, $^3J_{1'\text{-}2'}$ = 9.0 Hz); 5.36 (m, 1H, $\mathbf{H_{3'}}$); 4.97 (m, 1H, POCH(CH_3)); 4.16 (m, 1H, $\mathbf{H_{4'}}$); 3.90 (m, 2H, 2x$\mathbf{H_{5'}}$); 3.22 (ABX, 2H, POCH(CH_3)$\mathbf{CH_2}$, $^2J_{A\text{-}B}$ = 16.1 Hz, $^3J_{B\text{-}X}$ = 2.0 Hz, $^3J_{A\text{-}X}$ = 11.3 Hz); 2.49 (B of ABX, 1H, $\mathbf{H_{2'b}}$, $^2J_{2'b\text{-}2'a}$ = 13.6 Hz; $^3J_{2'b\text{-}1'}$ = 4.9 Hz); 2.18 (A of ABX, 1H, $\mathbf{H_{2'a}}$, $^2J_{2'a\text{-}2'b}$ =

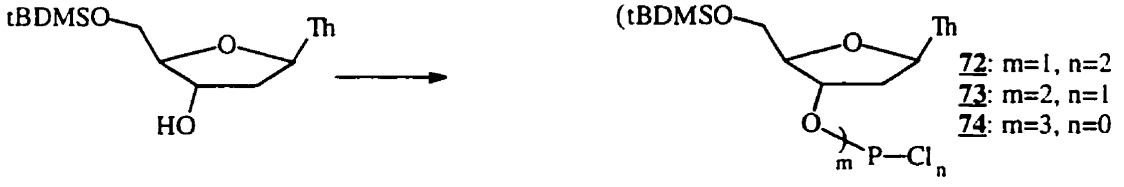
13.6 Hz, $^3J_{2'a\text{-}1'}$ = 9.0 Hz); 1.90 (s, 3H, CH=C(C**H**$_3$); 1.60 (d, 3H, POCH(C**H**$_3$), $^3J_{H\text{-}H}$ = 5.9 Hz); 0.90 (s, 9H, SiC(C**H**$_3$)$_3$); 0.15 (s, 6H, Si(C**H**$_3$)$_2$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 164.15 (N**C**=OC(CH$_3$)); 151.18 (N**C**=ONH); 148.21 (N=**C**NP); 135.27 (N**C**=C(CH3)); 131.21 (d, NC=**C**NP, $^2J_{C\text{-}P}$ = 12.5 Hz); 118.24 (d, N**C**=CNP, $^3J_{C\text{-}P}$ = 6.1 Hz); 114.86 (NC=**C**(CH$_3$)); 87.28 (d, **C**$_{4'}$, $^3J_{C4'\text{-}P}$ = 4.2 Hz); 85.95 (**C**$_{1'}$); 76.45 (d, POCH(CH$_3$), $^2J_{C\text{-}P}$ = 5.1 Hz); 67.36 (d, **C**$_{3'}$, $^2J_{C3'\text{-}P}$ = 6.1 Hz); 64.23 (**C**$_{5'}$); 41.28 (d, **C**$_{2'}$, $^3J_{C2'\text{-}P}$ = 3.9 Hz); 35.28 (d, POCH(CH$_3$)**C**H$_2$, $^3J_{C\text{-}P}$ = 6.9 Hz); 27.12 (SiC(**C**H$_3$)$_3$); 23.95 (POCH(**C**H$_3$)); 19.45 (SiC(CH$_3$)$_3$); 12.54 (CH=C(**C**H$_3$)); -5.62, -5.69 (Si(**C**H$_3$)$_2$);

^{31}P NMR (109.38 MHz, $CDCl_3$) δ 53.1;

MS (FAB, NBA) m/z 543 (11.9%, $(M+H)^+$); 339 (44.2%, (5'-O-tBDMS-thymidine+1-H_2O)).

Attempts to the synthesis of (5'--*ter*butyldimethylsilyl)thymid-3'-yl dichloro-phosphite 72



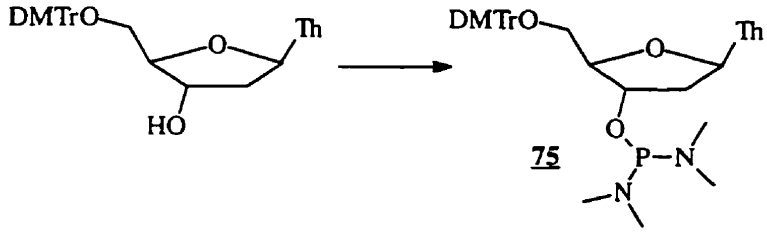
Typical procedure:

To a solution of freshly distilled phosphorus trichloride (17 µl, 0.20 mmol) in 10 ml dry dichloromethane cooled down to 0°C and stirred under Ar, was added a solution of 5'-O-tBDMS-thymidine (71 mg, 0.2 mmol) and dry diisopropylethylamine (87 µl, 0.50 mmol) in 2 ml dry dichloromethane, over 2h. After the addition, an aliquot was syringed out and introduced into a dry NMR tube equipped with a septum and flushed with Ar. 31P NMR was recorded and indicated the presence of 3 signals at 177.0, 165.1 and 154.4 ppm, attributed respectively to 72, 73 and 74. No significant change was obtained after 4 h or after 16h stirring at RT, except the presence of hydrolysis products around 0-15 ppm. In other experiments, the temperature was varied from -78°C to 40°C, the solvent was

replaced by dry diethyl ether, the base was changed for triethylamine, the length of introduction of the nucleoside was increased, but never could a single product be obtained.

When 5'-O-tBDMS-3-methyl thymidine was used, the results were essentially similar, indicating that the NH at position 3 of thymidine played no or little role in the process.

Synthesis of bis(dimethylamino)-(5'-(4,4'-dimethoxy)trityl-thymid-3'-yl)phosphoro-diamidite 75

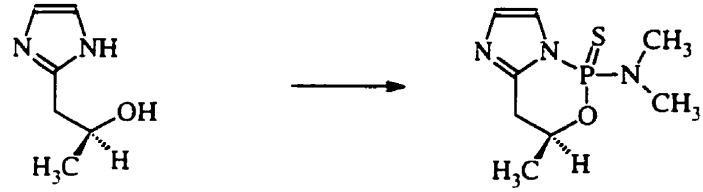


In a scrupulously dry glassware, to a solution of hexamethylphosphorous triamide HMPT (36 µl, 0.2 mmol) in 5 ml dry THF stirred at 70°C and containing an Ar inlet so that the Ar bubbles into the solution, a solution of 5'-O-DMTR-thymidine (109 mg, 0.2 mmol) in 1 ml dry dichloromethane was added dropwise over 1h. After 30 min., the ^{31}P NMR signal corresponding to HMPT at 122.7 ppm completely disappeared and gave rise to a signal corresponding to the desired phosphorodiamidite at 136.0 ppm. The mixture was allowed to cool down to ambient temperature and was evaporated *in vacuo*. Upon attempts to purify this compound by column chromatography, most of it decomposed. However, a reasonably pure sample was obtained directly after the reaction had been evaporated.

^1H NMR (270 MHz, $CDCl_3$) δ 8.12 (b, 1H, $N_3\mathbf{H}$); 7.56 (d, 1H, $\mathbf{H_6}$, $^4J_{H\text{-}H}$ = 1.2 Hz); 7.42-6.80 (m, 13H, aromatic **H**); 6.34 (dd, 1H, $\mathbf{H_{1'}}$, $^3J_{1'\text{-}2'}$ = 5.7 Hz, $^3J_{1'\text{-}2''}$ = 8.4 Hz); 4.42 (m, 1H, $\mathbf{H_{3'}}$); 4.10 (m, 1H, $\mathbf{H_{4'}}$); 3.88 (B of ABX, 1H, $\mathbf{H_{5'b}}$, $^2J_{5'b\text{-}5'a}$ = 11.4 Hz, $^3J_{5'b\text{-}4'}$ = 2.0 Hz); 3.74 (A of ABX, 1H, $\mathbf{H_{5'a}}$, $^2J_{5'a\text{-}5'b}$ = 11.4 Hz, $^3J_{5'a\text{-}4'}$ = 3.4 Hz); 3.81 (s, 6H, 2x$OC\mathbf{H_3}$); 2.54, 2.51 (2d, 12H, 2x$N(C\mathbf{H_3})_2$, $^3J_{H\text{-}P}$ = 5.7 Hz); 2.54-2.50 (m, 1H, $\mathbf{H_{2'}}$, overlap with $N(CH_3)_2$); 1.98 (m, 1H, $\mathbf{H_{2''}}$); 1.88 (d, 3H, $C_5(\mathbf{CH_3})$, $^4J_{H\text{-}H}$ = 1.2 Hz); 0.90 (s, 9H, $SiC(\mathbf{CH_3})_3$); 0.09 (s, 6H, $Si(\mathbf{CH_3})_2$);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 136.0.

Synthesis of (7S)-5-N,N-dimethylamino-7-methyl-5-thio-imidazo[3,4-a]oxazaphosphorine 79



54 79

To a solution of freshly distilled HMPT (182 µl, 1.0 mmol) in 10 ml dry acetontrile stirred at 65°C under Ar, with an Ar inlet bubbling inside through a needle, was introduced a solution of imidazolylpropanol **54** (126 mg, 1.0 mmol) in 2 ml dry pyridine. Aliquots were syringed out of the solution, and introduced into a dry NMR tube equipped with a septum, flushed with Ar. After 30 min, ^{31}P NMR indicated essentially only the presence of HMPT. After 20h only, this signal had completely disappeared, giving rise to two signals at 127.4 and 103.8 ppm respectively, in a ratio of 1:2. Several hydrolysis products were also observed, accounting for about 30% of the total amount of the mixture. Beaucage's sulfurizing reagent (200 mg, 1.0 mmol) was then introduced into the mixture, and after 30 min 2 signals were observed at 81.8 and 62.4 ppm, in a ratio of 2:1, as well as about 30% of products between 0 and 55 ppm, which is an area where usually H-phosphonates (0-20 ppm) as well as their sulfurized derivatives (50-55 ppm) are found. The solvents were removed *in vacuo*, and the mixture was loaded on a silica gel column, eluted with ethyl acetate:triethylamine 80:20. A complex mixture of products was obtained, having resonance signals between 0 and 60 ppm by ^{31}P NMR.

The signals observed at 127.4, 103.8 were attributed to the imidazo-oxazaphosphorine derivative of trivalent phosphorus **78**, the signals at 81.8 and 62.4 ppm were attributed to the thio derivative **79**, by analogy to the synthesis of the analogous products **99** and **100** which showed an identical behavior but could be isolated.

Synthesis of bis-aryl (N,N-diisopropyl) phosphoramidites 98, 102, 104, 106

98: $R_1 = R_3 = H$, $R_2 = NO_2$
102: $R_1 = R_3 = H$, $R_2 = Cl$
104: $R_1 = R_3 = Cl$, $R_2 = H$
106: $R_1 = R_2 = Cl$, $R_3 = H$

Preparation of the substituted phenoxide salts

To 50 ml freshly distilled methanol stirred at 0°C under Ar, was added sodium metal (115 mg, 5 mmol). After 30 min., all the sodium had reacted with methanol, producing sodium methoxide and gaseous hydrogen. To this solution maintained at 0°C was added the substituted phenol (previously dried by double azeotroping with dry benzene then drying *in vacuo* overnight): either 4-chlorophenol (707 mg, 5.5 mmol), 4-nitrophenol (765 mg, 5.5 mmol), 2,6-dichlorophenol (896 mg, 5.5 mmol) or 2,4-dichlorophenol (896 mg, 5.5 mmol) in one portion. After 30 min. stirring at 0°C and 1 h at RT, the solvent was evaporated in vacuo, yielding a salt. This salt was washed twice with 5 ml cold dry THF, to remove the excess unreacted phenol. It was then azeotroped twice with dry benzene then dried *in vacuo* overnight.

Preparation of phosphoramidite derivatives 98, 102, 104, 106

To a suspension of the substituted sodium phenoxide salt (3.0 mmol, 452 mg of sodium 4-chlorophenoxide, 483 mg of sodium 4-nitrophenoxide, 555 mg of sodium 2,6-dichlorophenoxide or 555 mg of sodium 2,4-dichlorophenoxide) in 10 ml dry THF stirred under Ar at 0°C was added N,N-diisopropylphosphoramidous dichloride (464 µl, 3.3 mmol). After 30 min., ^{31}P NMR indicated the presence of a single signals at 144.8, 145.3, 153.6 and 147.2 ppm, corresponding respectively to **98**, **102**, **104** and **106**.

The crude mixture was then percolated through a 5 cm pad of silica gel and washed with 50 ml dry THF. Evaporation of the solvent *in vacuo* yielded either a white

sticky solid or a colorless oily compound that solidified to sticky solids upon standing or upon the addition of a small quantity of dry acetonitrile followed by evaporation.

bis-(4-nitrophenyl) N,N-diisopropyl phosphoramidite 98

^{1}H NMR (270 MHz, $CDCl_3$) δ 8.14 (d, BB' of AA'BB',4H, $(NO_2)C=CH$, $^3J_{H-H}$ = 9.1 Hz); 7.10 (dd, AA' of AA'BB', 4H, $OC=CH$, $^3J_{H-H}$ = 9.1 Hz, $^4J_{H-P}$ = 1.7 Hz); 3.70 (dh, 2H, $NCH(CH_3)_2$, $^3J_{H-H}$ = 6.7 Hz, $^3J_{H-P}$ = 11.6 Hz); 1.23 (d, 12H, $NCH(CH_3)_2$, $^3J_{H-H}$ = 6.7 Hz);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 159.50 ($C(NO_2)$); 143.10 (OC); 125.86 ($(NO_2)C=CH$); 119.59 (d, $OC=CH$, $^3J_{C-P}$ = 9.3 Hz); 44.70 (d, $NCH(CH_3)_2$, $^2J_{C-P}$ = 12.9 Hz); 24.53 ($NCH(CH_3)_2$);

^{31}P NMR (109.38 MHz, $CDCl_3$) δ 144.5;

MS (CI) m/z 408 (47.7%, $(M+H)^+$); 269 (100.0%, $(M-(NO_2)C_6H_4O)$).

bis-(4-chlorophenyl) N,N-diisopropyl phosphoramidite 102

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.22 (b, 4H, $ClC=CH$); 6.97 (b, 4H, $OC=CH$); 3.82 (m, 2H, $NCH(CH_3)_2$); 1.20 (d, 12H, $CH(CH_3)_2$, $^3J_{H-H}$ = 5.9 Hz);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 145.3;

MS (CI) m/z 386 (8.0%, $(M+H)^+$); 258 (100.0%, $(M(^{35}Cl)-OC_6H_4Cl)$), 260 (32.9% $(M(^{37}Cl)-OC_6H_4Cl)$).

bis-(2,6-dichlorophenyl) N,N-diisopropylphosphoramidite 104

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.22 (m, 4H, H_{meta}); 6.87 (m, 2H, H_{para}); 4.12 (m, 2H, $NCH(CH_3)_2$); 1.31 (d, 12H, $NCH(CH_3)_2$, $^3J_{H-H}$ = 6.5 Hz);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 147.35 (C_{ortho}); 128.81 (C_{meta}); 128.57 (d, $POC=C$, $^2J_{C-P}$ = 2.1 Hz); 123.89 (C_{para}); 44.83 (d, $NCH(CH_3)_2$, $^2J_{C-P}$ = 15.0 Hz); 24.71 (d, $NCH(CH_3)_2$, $^3J_{C-P}$ = 13.2 Hz);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 153.5;

MS (CI) m/z 456 (3.7%, $(M+H)^+$); 292 (100.0%, $(M-OC_6H_3Cl_2)$).

bis-(2,4-dichlorophenyl) N,N-diisopropylphosphoramidite 106

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.45 (s, 2H, $ClC=CHCCl$); 7.27-6.90 (m, 4H, $OCCH=CH$); 3.90 (dh, 2H, $NCH(CH_3)_2$, $^3J_{H-H}$ = 6.9 Hz, $^3J_{H-P}$ = 11.1 Hz); 1.25 (d, 12H, $NCH(CH_3)_2$, $^3J_{H-H}$ = 6.9 Hz);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 151.0, 149.7, 129.9, 127.5, 120.3, 116.4 (aromatic C); 45.3 (d, $NCH(CH_3)_2$, $^2J_{C\text{-}P}$ = 13.7 Hz); 24.1 (d, $NCH(CH_3)_2$, $^3J_{C\text{-}P}$ = 9.5 Hz);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 147.2;

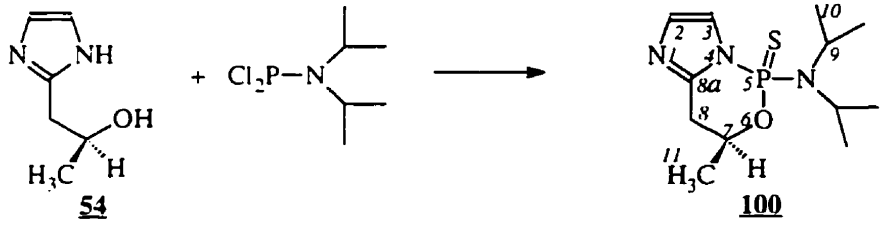
MS (CI) m/z 456 (1.2%, $(M+H)^+$); 292 (100.0%, $(M\text{-}OC_6H_3Cl_2)$).

Acid-catalyzed reaction of the phosphoramidites with isopropanol

to a solution of the phosphoramidite (203 mg of **98**, 193 mg of **102**, 228 mg of **104** or 228 mg of **106**, 0.5 mmol) in dry THF (5 ml) stirred under Ar at RT was added a solution of isopropanol (43 µl, 0.55 mmol) and tetrazole (175 mg, 2.5 mmol) in 2 ml dry THF. Aliquots were syringed out of the mixture, and introduced into a dry NMR tube equipped with a septum, flushed with Ar and ^{31}P NMR spectra were recorded. The signals for **98**, **102**, **104** and **106** appeared respectively at 144.8, 145.3, 153.6 and 147.2 ppm. After 3h, only **102** had reacted completely, giving rise to a signal at 129.2 ppm. **98** did not show any reaction after 16, nor did **104**. Phosphoramidite **106** showed some trace of a product at 135.0 ppm, which increased to about 20% after 16h.

Synthesis of (7*S*)-7,8-dihydro-5-(N,N-diisopropylamino)-7-methyl-5-thio-imidazo [3,4-a]oxazaphosphorine 100

Method A: from diisopropylphosphoramidous dichloride

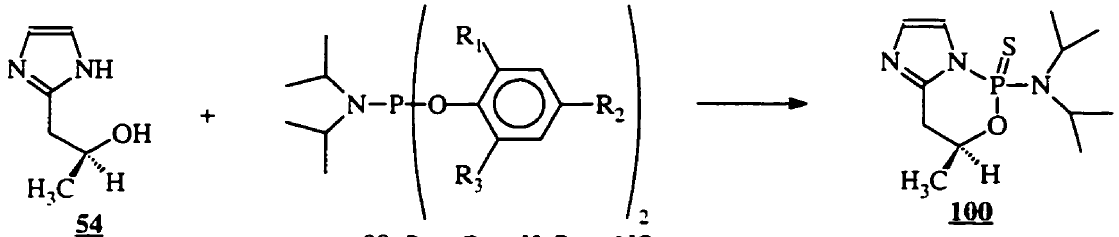


54 **100**

To a solution of dry N,N-diisopropylethylamine (261 µl, 1.5 mmol) in deuteriochloroform kept at 0°C, was added N,N-diisopropylphosphoramidous dichloride (42 µl, 0.3 mmol). The tube was shaken, and after 15 min. ^{31}P NMR indicated the presence of two signals at 126.0 and 103.8 ppm in a ratio of 2:1 respectively. After 3h at RT no change was observed, as well as after 2h at 50°C. To the mixture was then added elemental sulfur (11 mg, 0.33 mmol). Complete sulfurization required 3h to occur, giving

two compounds having resonance frequencies at 58.8 and 51.9 ppm in a ratio of 2:1 respectively. The solvent was evaporated, and the mixture of diastereomers was isolated from the reaction mixture by flash chromatography (ethyl acetate:hexanes:triethylamine 80:10:10). The fast-eluting diastereomer could be separated from the mixture by column chromatography (ethyl acetate:hexanes:triethylamine 70:25:5) in 50.0% yield (43 mg), and then a mixture of the two diastereomers was obtained in 46.2% yield (40.0 mg).

Method B: from bis-aryl N,N-diisopropyl phosphoramidites 98, 104 or 106



98: $R_1 = R_3 = H$, $R_2 = NO_2$
104: $R_1 = R_3 = Cl$, $R_2 = H$
106: $R_1 = R_2 = Cl$, $R_3 = H$

In a dry NMR tube under Ar, into a solution of (S)-1-(imidazol-1-yl)propan-2-ol **54** (38 mg, 0.3 mmol) and bis-aryl N,N-diisopropylphosphoramidite (122 mg of **98**, 136 mg of **104** or **106**, 0.3 mmol) in 0.5 ml dry THF was syringed DBU (100 µl, 0.66 mmol). The tube was shaken, then ^{31}P NMR was recorded and showed the appearance of two signals at 125.9 and 103.6 ppm in a ratio of 1.5:1 to 2.5:1. The mixture was sulfurized slowly as previously.

fast-eluting isomer

^1H NMR (500 MHz, $CDCl_3$) δ 7.03 (d, 1H, $\mathbf{H_3}$, $^3J_{3\text{-}2}$ = 1.7 Hz); 7.00 (d, 1H, $\mathbf{H_2}$, $^3J_{2\text{-}3}$ = 1.7 Hz); 4.99 (dddq, 1H, $\mathbf{H_7}$, $^3J_{7\text{-}8a}$ = 11.7 Hz, $^3J_{7\text{-}8b}$ = 2.7 Hz, $^3J_{7\text{-}11}$ = 6.8 Hz, $^3J_{7\text{-}P}$ = 2.4 Hz); 3.64 (dh, 1H, $\mathbf{H_9}$, $^3J_{9\text{-}10}$ = 6.8, $^3J_{9\text{-}P}$ = 21.7 Hz); 3.10 (m, B of ABX, 1H, $\mathbf{H_{8b}}$); 2.90 (A of ABX, 1H, $\mathbf{H_{8a}}$, $^2J_{8a\text{-}8b}$ = 16.8 Hz, $^3J_{8a\text{-}7}$ = 11.7 Hz); 1.51 (d, 3H, $\mathbf{H_{11}}$, $^3J_{11\text{-}7}$ = 6.8 Hz); 1.29 (d, 6H, $\mathbf{H_{10}}$, $^3J_{10\text{-}9}$ = 6.8 Hz); 1.27 (d, 6H, $\mathbf{H_{10}}$, $^3J_{10\text{-}9}$ = 6.8 Hz);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 147.15 (s, $\mathbf{C_{8a}}$); 129.75 (d, $\mathbf{C_3}$, $^2J_{C3\text{-}P}$ = 13.3 Hz); 117.12 (d, $\mathbf{C_2}$, $^3J_{C2\text{-}P}$ = 8.2 Hz); 72.48 (d, $\mathbf{C_7}$, $^2J_{C7\text{-}P}$ = 6.0 Hz); 48.17 (d, $\mathbf{C_9}$, $^2J_{C9\text{-}P}$ = 5.5 Hz); 33.78 (s, $\mathbf{C_8}$); 22.28 (d, $\mathbf{C_{10}}$, $^2J_{C10\text{-}P}$ = 1.4 Hz); 22.24 (s, $\mathbf{C_9}$); 21.72 (d, C_{11}, $^3J_{C11\text{-}P}$ = 11.4 Hz);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 58.8;

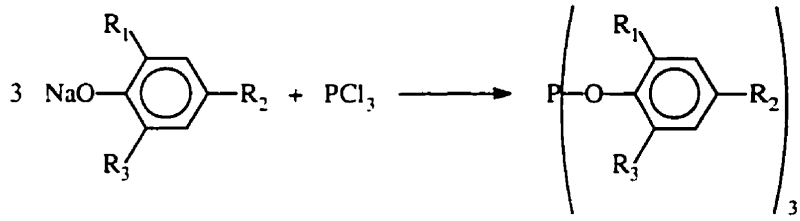
MS (EI) m/z 287 (51.6%, $M^{+\bullet}$); 244 (34.5%, (M-CH_3CHCH_3)); 187 (25.7%, (M-$NCH(CH_3)_2$)); 109 (100.0% ($ImCH_2CHCH_3$))

slow-eluting isomer

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.01 (d, 1H, **H$_3$**, $^3J_{3-2}$ = 1.5 Hz); 6.91 (d, 1H, **H$_2$**, $^3J_{2-3}$ = 1.5 Hz); 4.79 (m, 1H, **H$_7$**); 3.42 (dh, 1H, **H$_9$**, $^3J_{9-10}$ = 6.7 Hz, $^3J_{9-P}$ = 15.1 Hz); 3.03 (B of ABX dedoubled, 1H, **H$_{8b}$**, $^2J_{8b-8a}$ = 16.8 Hz, $^3J_{8b-7}$ = 2.7; $^4J_{8b-P}$ = 1.5 Hz); 2.96 (A of ABX dedoubled, 1H, **H$_{8a}$**, $^2J_{8a-8b}$ = 16.8 Hz, $^3J_{8a-7}$ = 5.9 Hz, $^4J_{8a-P}$ = 1.2 Hz); 1.44 (dd, 3H, **H$_{11}$**, $^3J_{11-7}$ = 5.9 Hz, $^3J_{11-P}$ = 1.5 Hz); 1.21 (d, 6H, **H$_{10}$**, $^3J_{10-9}$ = 6.6 Hz); 1.19 (d, 6H, **H$_{10}$**, $^3J_{10-9}$ = 6.8 Hz);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 51.9.

Synthesis of triaryl phosphites <u>101</u>, <u>108</u>, <u>109</u>



<u>101</u>: $R_1 = R_3 = H$, $R_2 = NO_2$
<u>108</u>: $R_1 = R_2 = Cl$, $R_3 = H$
<u>109</u>: $R_1 = R_3 = Cl$, $R_2 = H$

The preparation of sodium salts of substituted phenoxides is reported on p. 132.

To a suspension of the substituted sodium phenoxide salt (3.0 mmol, 483 mg of sodium 4-nitrophenoxide, 555 mg of sodium 2,6-dichlorophenoxide or 555 mg of sodium 1,4-dichlorophenoxide) in 10 ml dry THF stirred under Ar at 0°C was added freshly distilled phosphorus trichloride (288 µl, 3.3 mmol). After 1h, ^{31}P NMR indicated the presence of a single signal at 125.7, 129.0 or 141.7 ppm corresponding to the presence of **<u>101</u>**, **<u>108</u>** or **<u>109</u>** respectively. The solution was then percolated through a 5 cm pad of silica gel and washed with 50 ml dry THF. After evaporation of the solvent, the ^{31}P NMR of **<u>101</u>** indicated a mixture of compounds having resonance signals between 126 and 0 ppm, signifying that the compound had decomposed at some point in the purification process. However, phosphite triesters **<u>108</u>** and **<u>109</u>** could be obtained as colorless oils that crystallized upon standing to sticky white solids.

tris-(2,4-dichlorophenyl) phosphite 108

^{1}H NMR (270 MHz, CDCl3) δ 7.53 (s, 3H, ClC=CHCCl); 7.35-7.00 (m, 6H, OCCH=CH);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 153.40 (b), 147.56 (b), 128.32 (b), 127.61 (b), 120.96 (b), 116.5 (b);

^{31}P NMR (109.38 MHz, $CDCl_3$) δ 128.5;

MS (EI) m/z 516 (11.8%, $M^{+\bullet}$, including 5 ^{35}Cl and 1 ^{37}Cl); 514 (11.4%, $M^{+\bullet}$, including 6 ^{35}Cl); 355 (100.0%, (M-$OC_6H_3Cl_2$), including 3 ^{35}Cl and 1 ^{37}Cl); 357 (48.2%, (M-$OC_6H_3Cl_2$), including 2 ^{35}Cl and 2 ^{37}Cl).

tris-(2,6-dichlorophenyl) phosphite 109

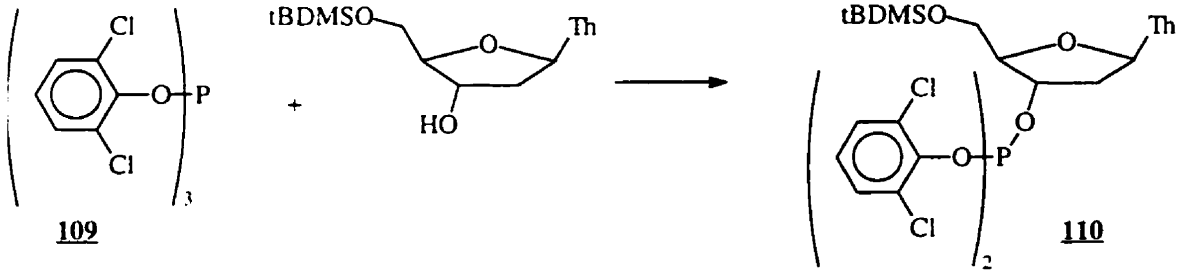
^{1}H NMR (270 MHz, $CDCl_3$) δ 7.31 (m, 6H, $\mathbf{H_{meta}}$); 6.95 (m, 3H, $\mathbf{H_{para}}$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 131.06, 128.54 (b), 128.12 (d, $J_{C\text{-}P}$ = 12.3 Hz); 125.62 (d, $J_{C\text{-}P}$ = 7.6 Hz);

^{31}P NMR (109.38 MHz, $CDCl_3$) δ 141.5;

MS (EI) m/z 516 (8.5%, $M^{+\bullet}$); m/z 518 (7.0%, (M+2)); 355 (100.0%, (M-$C_6H_3{}^{35}Cl_2O$)); 353 (77.90%, (M-$C_6H_3{}^{37}Cl{}^{35}ClO$)).

Synthesis of bis(2,6-dichloro)phenyl (5'-O-*ter*butyldimethylsilyl)thymid-3'-yl phosphite 110



To a turbid mixture of tris(2,6-dichlorophenyl) phosphite **109** (517 mg, 1.0 mmol) and 5'-O-tBDMS-thymidine (356 mg, 1.0 mmol) in 20 mmol of scrupulously dry THF cooled down to -20°C and stirred under Ar, was added a solution of dry DBU (150 µl, 1.0 mmol) in 5.0 ml dry THF, over a period of time of 2h. The mixture was then allowed to warm up to room temperature and 0.5 g of silica gel was added, then it was dried *in vacuo*

until a white solid was obtained. This solid residue was then loaded on a column and the desired compound was isolated by flash chromatography using hexanes:ethyl acetate (previously washed twice with a saturated sodium bicarbonate solution and dried over magnesium sulfate) as an eluent (60:40).

The compound was then evaporated *in vacuo* to a colorless, that crystallized upon standing. This compound was moisture sensitive but could however be stored for several weeks in scrupulously dry conditions. The yield was relatively low, due to two factors. On one hand, during the reaction an unwanted side product was synthesized as well (δ 138.2 ppm by ^{31}P NMR) in a ratio of 1:3 with the desired compound (δ 130.2 ppm). This compound was assigned to the substitution of two dichlorophenyl moieties by 5'-O-tBDMS-thymidine. However, the unwanted side product decomposed during chromatography. On the other hand, the desired compound tended to decompose during chromatography, regardless of the precautions used. The desired product was obtained in 57.3 % yield, m = 407 mg of a white solid, m.p. 75-77°C (dec.).

^{1}H NMR (assignments based on COSY experiment) (270 MHz, $CDCl_3$) δ 8.85 (s, 1H, N**H**); 7.60 (s, 1H, $\mathbf{H_6}$); 7.20-7.40 (m, 4H, aromatic **H** *meta* to the oxygen); 6.90-7.00 (m, 2H, aromatic **H** *para* to the oxygen); 6.45 (dd, 1H, $\mathbf{H_{1'}}$, $^{3}J_{1'\text{-}2'b} = 5.1$ Hz; $^{3}J_{1'\text{-}2'a} = 9.4$ Hz); 5.85 (m, 1H, $\mathbf{H_{3'}}$); 4.45 (m, 1H, H4'); 3.95 (AB of ABX, 2H, $\mathbf{H_{5'a}}$, $\mathbf{H_{5'b}}$; $^{2}J_{5'a\text{-}5'b} = 11.4$ Hz; $^{3}J_{5'a\text{-}4'} = 2.0$ Hz; $^{3}J_{5'b\text{-}4'} = 1.5$ Hz); 2.65 (dd, 1H, $\mathbf{H_{2'b}}$, $^{2}J_{2'b\text{-}2'a} = 13.6$ Hz; $^{3}J_{2'b\text{-}1'} = 5.1$ Hz); 2.20 (m, 1H, $\mathbf{H_{2'a}}$); 1.87 (s, 3H, C=CC**H**$_3$); 0.85 (s, 9H, C(C**H**$_3$)$_3$); 0.10 (s, 6H, Si(C**H**$_3$)$_2$);

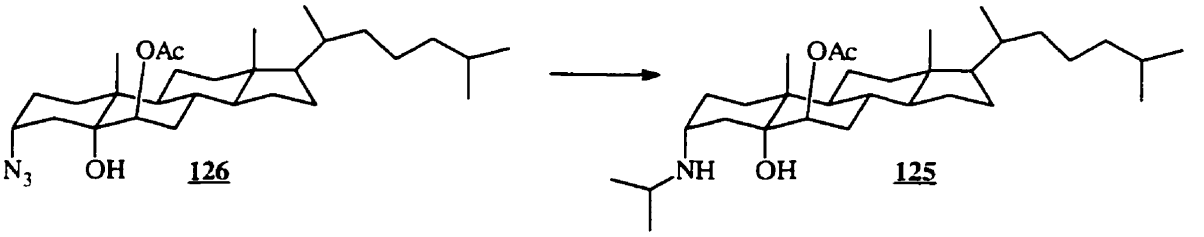
^{13}C NMR (assignments based on APT and HETCOR experiments) (67.94 MHz, $CDCl_3$) δ 163.87 ($\mathbf{C_4}$); 150.39 ($\mathbf{C_2}$); 135.43 ($\mathbf{C_6}$); 129.0, 128.33, 128.12 (d, $J_{C\text{-}P} = 14.5$ Hz); 125.22 (d, $J_{C\text{-}P} = 8.3$ Hz); 110.99 ($\mathbf{C_5}$); 86.89 ($\mathbf{C_{4'}}$); 84.98 ($\mathbf{C_{1'}}$); 75.92 (d, $\mathbf{C_{3'}}$, $^{2}J_{C\text{-}P} = 5.2$ Hz); 63.34 ($\mathbf{C_{5'}}$); 40.77 ($\mathbf{C_{2'}}$); 26.01 (C(C**H**$_3$)$_3$); 18.37 (**C**(CH$_3$)$_3$); 12.56 (C=C(**C**H$_3$)); -5.26, -5.36 (Si(C**H**$_3$)$_2$);

^{31}P NMR (121.0 MHz, $CDCl_3$) δ 131.3;

MS (FAB, NBA) m/z 711 (3.7%, $(M+H)^+$); 585 (10.7%, (M - thymine)); 339 (100%, (5'-O-tBDMS-thymidine + 1 - H_2O)).

6.3. Experimentals for section 3

Synthesis of 6β-acetoxy-5-hydroxy-3α-(N-isopropylamino)cholest-5α-ane 125



A solution of azide **126** (300 mg, 0.6 mmol) and Pd/C (150 mg of a 10% mixture Pd/C) in 20 ml of ethanol was shaken at RT under 40 psi of hydrogen for 16h. TLC then indicated that all the starting azide had been consumed. The mixture was filtered over a short compact pad of celite, and evaporated *in vacuo* to a yellow oily residue, which was left *in vacuo* overnight. The intermediate amine was not purified, however its MS was recorded and gave satisfactory results. The crude mixture was then dissolved in 10 ml dry methanol, and the pH of the reaction mixture was adjusted to 5.5 by slow addition of acetic acid. Acetone (440 µl, 6 mmol) was then added, followed by sodium cyanoborohydride (189 mg, 3 mmol). The pH of the mixture was maintained to 5.5 and it was stirred for 24h at RT under Ar. At the end of the reaction, 2 ml of a saturated solution of sodium bicarbonate were added and the stirring was maintained for 30 min. The mixture was then concentrated *in vacuo*, and taken up in 30 ml dichloromethane, washed twice with a mixture of 10% sodium carbonate solution and brine (3 ml of each), dried over magnesium sulfate and evaporated *in vacuo* to a sticky solid. This crude compound was purified by flash chromatography (hexanes:ethyl acetate 90:10 to hexanes:ethyl acetate:triethylamine 80:10:10), and yielded 217 mg (72% from azide **132**) of pure amine **125**, m.p. 80-82°C.

^{1}H NMR (270MHz, $CDCl_3$) δ 4.74 (m, 1H, $\mathbf{H_{6e}}$); 3.24(m, 1H, $\mathbf{H_{3e}}$); 2.96 (h, 1H, $NC\mathbf{H}(CH_3)_2$ $^3J_{H\text{-}H}$ = 6.7 Hz); 2.03 (s, 3H, $OCOC\mathbf{H_3}$); 2.00-0.70 (several multiplets, $\mathbf{H_1}$-$\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_7}$-$\mathbf{H_{27}}$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 169.60 (**C**=O); 76.56 ($\mathbf{C_6}$); 73.76 ($\mathbf{C_5}$); 55.53, 55.11, 49.11, 44.76, 44.52, 42.06, 39.28, 38.84, 38.77, 35.51, 35.22, 30.85, 30.33, 30.02, 28.31,

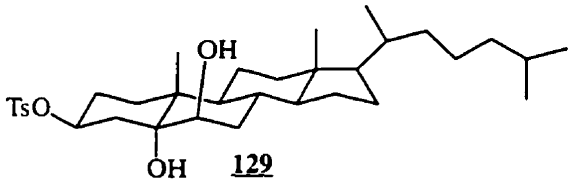
27.60, 27.35, 26.20, 17.99, 15.58, 11.52, 23.44, 23.29, 22.18, 21.91, 21.22, 20.87, 20.03 ($\mathbf{C_1}$-$\mathbf{C_4}$, $\mathbf{C_7}$-$\mathbf{C_{27}}$, CH_3CO, $NHCH(CH_3)_2$);

MS (intermediate amine, CI) m/z 462 (100.0%, $(M+H)^+$); 444 (26.6%, $(M+1-H_2O)^{+\bullet}$); 402 (16.0%, $(M+1-CH_3CO_2H)^{+\bullet}$); 384 (34.0%, $(M-NH_3-CH_3CO_2H)^{+\bullet}$);

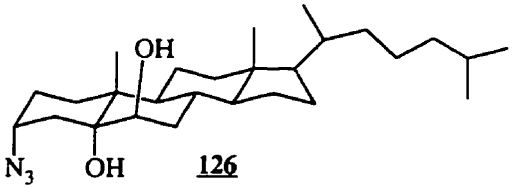
MS (N-isopropyl derivative, EI) m/z 503 (62.6%, $M^{+\bullet}$); 488 (85.5%, $(M+1-H_2O)$); 98 (100.0%);

$[\alpha]^D_{295}$ -44.9 (c 1.0, ethyl acetate).

Synthesis of 3α-azido-5,6β-dihydroxycholest-5α-ane <u>126</u>



<u>129</u>



<u>126</u>

To a solution of 3-tosyl-3β,5,6β-trihydroxycholest-5α-ane **<u>129</u>** (574 mg, 1.0 mmol) in 5 ml dry DMF stirred under Ar, was added lithium azide (98 mg, 2.0 mmol). The mixture was warmed up to 100°C and stirred under Ar at that temperature for 1.5 h. It was then allowed to cool down slowly to RT and was concentrated *in vacuo*. The mixture was then dissolved in 80 ml of dichloromethane and washed twice with 10 ml water and once with a saturated solution of sodium chloride. The organic layer was then dried on magnesium sulfate and evaporated *in vacuo* to 435 mg (98 % yield) of an off-white solid, m.p. 95-97°C.

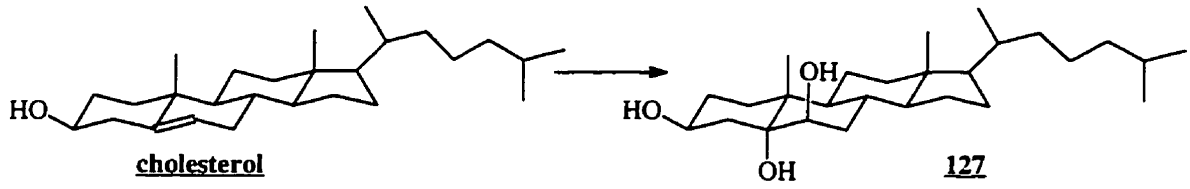
^{1}H NMR (200 MHz, $CDCl_3$) δ 4.07 (m, 1H, $\mathbf{H_3}$); 3.57 (m, 1H, $\mathbf{H_6}$); 3.45 (b, **OH**); 2.40-0.70 (several multiplets, 45H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_7}$-$\mathbf{H_{27}}$);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 75.26 ($\mathbf{C_6}$); 74.17 ($\mathbf{C_5}$); 58.39, 56.17, 55.75, 45.02, 42.70, 39.89, 39.48, 38.90, 36.13, 35.81, 34.31, 33.81, 30.09, 28.42, 28.21, 27.99, 25.00, 24.10, 23.86, 22.81, 22.54, 20.63, 18.63, 16.71, 12.14 ($\mathbf{C_1}$-$\mathbf{C_3}$, $\mathbf{C_4}$, $\mathbf{C_7}$-$\mathbf{C_{27}}$);

MS (EI) m/z 445 (5.2%, $M^{+\bullet}$); 427 (24.7%, $(M-H_2O)$); 399 (86.2%, $(M-H_2O-HN_3)$);

$[\alpha]^D_{295}$ -42.7° (c 1.65, CH_2Cl_2).

Synthesis of 3β,5,6β–trihydroxycholest-5α-ane 127



cholesterol 127

A solution of cholesterol (5 g, 12.9 mmol) in 50 ml 88% formic acid was heated up to 75-80°C and swirled for about 30 min. The mixture turned brown and an upper oily brown layer separated (cholesteryl formate). The mixture was cooled down to 10°C and 5 ml of a 30% solution of hydrogen peroxide was added slowly under stirring. After 10 min., the temperature slowly rose up to 35-40°C then came down to RT. After 3h, all the cholesterol in suspension had disappeared and the solution was clear. It was stirred at RT for 12h. Then, 10 ml of boiling water was added and a white precipitate formed. The solution was cooled down with an ice bath and the precipitate filtered with a büchner funnel. The solid residue was dissolved in 120 ml methanol, and to this solution was added 4 ml of a 25% (m/v) solution of sodium hydroxide. The solution was refluxed for 30 min. and cooled down to 0°C then 300 ml of cold water was added. An abundant white precipitate formed, and the solution was brought to pH 3-4 with a normal solution of hydrochloric acid. The white solid was filtered, dried and recrystallized from methanol (m.p. 242-243°C) (lit.[205] 244°C).

^{1}H NMR (200 MHz, CD_3COCD_3) δ 4.0 (m. 1H, $\mathbf{H_3}\alpha$); 3.55 (m, 1H, $\mathbf{H_6}\alpha$); 2.20-0.75 (several multiplets., 43H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_7}$-$\mathbf{H_{27}}$);

^{13}C NMR (75.3 MHz, CD_3COCD_3) (assignments from Konno and Hikino[206]) δ 76.36 ($\mathbf{C_6}$); 76.05 ($\mathbf{C_5}$); 67.68 ($\mathbf{C_3}$); 56.86, 56.76 ($\mathbf{C_{14}}$, $\mathbf{C_{17}}$); 46.09 ($\mathbf{C_9}$); 43.21 ($\mathbf{C_{13}}$); 42.19 ($\mathbf{C_4}$); 40.73 ($\mathbf{C_{12}}$); 39.99 ($\mathbf{C_{24}}$); 39.15 ($\mathbf{C_{10}}$); 36.53 ($\mathbf{C_{22}}$); 36.31 ($\mathbf{C_{20}}$); 35.80 ($\mathbf{C_7}$); 33.21 ($\mathbf{C_2}$); 32.50 ($\mathbf{C_1}$); 31.39 ($\mathbf{C_8}$); 28.71 ($\mathbf{C_{16}}$); 24.66 ($\mathbf{C_{15}}$); 24.40 ($\mathbf{C_{23}}$); 22.94, 22.85 ($\mathbf{C_{26}}$, $\mathbf{C_{27}}$); 21.96 ($\mathbf{C_{11}}$); 19.19 ($\mathbf{C_{21}}$); 17.25 ($\mathbf{C_{19}}$); 12.60 ($\mathbf{C_{18}}$);

MS (EI) m/z 420 (5.4%, $M^{+\cdot}$), 402 (100.0%, ($M-H_2O$)), 384 (71.3%, ($M-2H_2O$));

$[\alpha]^D_{295}$ +2.1° (c 1.0, EtOAc).

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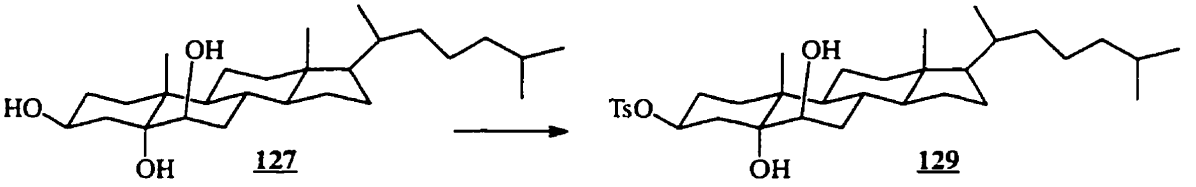
[205] Fieser, L.F.; Rajagopalan, S. *J. Am. Chem. Soc.* **1949**, *71*, 3938

[206] Konno, C.; Hikino, H. *Tetrahedron* **1976**, *32*, 325

Synthesis of 3-tosyl-3β,5,6β-trihydroxycholest-5α-ane 129



To a solution of 3β,5,6β-trihydroxycholest-5α-ane **127** (1.26 g, 3 mmol) in 30 ml dry pyridine was added freshly recrystallized p-toluenesulfonyl chloride (630 mg, 3.30 mmol) at RT under Ar. The mixture was stirred under Ar at RT for 40 h, then it was evaporated *in vacuo*. The residue was dissolved in 250 ml dichloromethane, washed twice with 30 ml of a 10% solution of ammonium chloride, then once with a saturated solution of sodium chloride. The organic layer was dried over magnesium sulfate and evaporated to an off-white crude product, which was purified by flash chromatography (ethyl acetate:petroleum ether 18:82) to afford 1.38 g (80.0 % yield) of pure product as a white solid, m.p. 163-164°C (dec.) (lit.[207] 166°C (dec.)).

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.78 (d, 2H, $\mathbf{H}_{ortho}$, $^3J_{ortho\text{-}meta}$ = 8.1 Hz); 7.31 (d, 2H, $\mathbf{H}_{meta}$, $^3J_{meta\text{-}ortho}$ = 8.1 Hz); 4.90 (m, 1H, $\mathbf{H}_3$); 3.46 (m, 1H, $\mathbf{H}_6$); 2.43 (s, 3H, $\mathbf{CH}_{3para}$); 2.30-0.70 (several multiplets, 45H, $\mathbf{H}_1$, $\mathbf{H}_2$, $\mathbf{H}_4$, $\mathbf{H}_7$-$\mathbf{H}_{27}$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 144.47, 134.63, 129.83, 127.70 (aromatic **C**); 80.42 ($\mathbf{C}_3$); 76.22 ($\mathbf{C}_6$); 75.87 ($\mathbf{C}_5$); 56.30, 55.88, 45.69, 42.78, 39.92, 39.56, 38.21, 38.17, 36.22, 35.84, 34.64, 32.29, 30.15, 28.26, 28.07, 27.71, 24.18, 23.93, 22.87, 22.62, 21.71, 21.15, 18.73, 16.72, 12.20 ($\mathbf{C}_1$, $\mathbf{C}_2$, $\mathbf{C}_4$, $\mathbf{C}_7$-$\mathbf{C}_{27}$, tosyl p- $\mathbf{CH}_3$);

MS (FAB, NBA+NaCl) m/z 575 (0.8%, $(M+H)^+$); 403 (14.2%, (M-TsOH)); 385 (100%, (M-TsOH-H_2O)); 367 (87.3%, (M-TsOH-2H_2O));

$[\alpha]^D_{295}$ -29.8° (c 1.3, EtOAc) (lit.[207] -27°).

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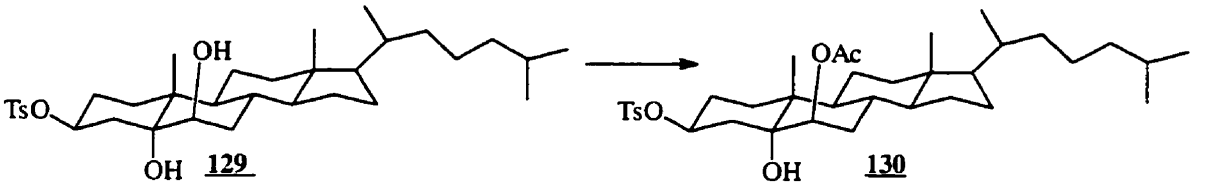
[207] Bourdon, S.; Ranisteano, S. *Bull. Soc. Chim. Fr.* **1960**, 1982

Synthesis of 6-acetyl-3-tosyl-3β,5,6β-trihydroxycholest-5α-ane 130



To a solution of 3-tosyl-3β,5,6β-trihydroxycholest-5α-ane **129** (574 mg. 1 mmol) in 5 ml dry pyridine stirred at 0°C under Ar was added 4-dimethylaminopyridine (122 mg, 1 mmol) and acetic anhydride (115 µl, 1.2 mmol). After 6 h, TLC indicated the reaction had gone to completion. Pyridine was evaporated *in vacuo*, then the oily residue was dissolved in 50 ml dichloromethane and washed twice with 5 ml of a 10% solution of ammonium chloride then 5 ml of brine. The organic layer was subsequently dried over magnesium sulfate and evaporated to a white solid, which was recrystallized from hexanes:ethyl acetate as a white solid, m = 555 mg (90% yield), m.p. 150°C (dec.) (lit.[208] 152°C).

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.76 (d, 2H, $\mathbf{H}_{ortho}$, $^3J_{ortho\text{-}meta}$ = 8.4 Hz); 7.31 (d, 2H, $\mathbf{H}_{meta}$, $^3J_{meta\text{-}ortho}$ = 8.4 Hz); 4.83 (m, 1H, $\mathbf{H_3}$); 4.57 (m, 1H, $\mathbf{H_6}$); 2.43 (s, 3H, $\mathbf{CH_3}C_6H_4$); 2.03 (s, 3H, CH_3COO); 2.00-0.64 (multiplets, 43H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_7}$-$\mathbf{H_{27}}$);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 170.00 (**C**=O); 144.55 (SO_2**C**=C); 129.80, 127.65 ($\mathbf{C}_{ortho}$, $\mathbf{C}_{meta}$); 79.64 ($\mathbf{C_3}$); 77.20 ($\mathbf{C_6}$); 75.75 ($\mathbf{C_5}$); 56.087, 55.70, 45.18, 42.66, 39.75, 39.47, 38.24, 37.74, 36.10, 35.75, 31.78, 31.23, 30.56, 28.16, 27.99, 27.59, 24.04, 23.80, 22.81, 22.54, 21.66, 21.37, 21.00, 18.62, 16.31, 12.14 ($\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_4}$, $\mathbf{C_7}$-$\mathbf{C_{27}}$, $COCH_3$, $\mathbf{CH_3}C_6H_4$);

MS (FAB, NBA + NaCl) m/z 639 (6.6%, $(M+Na)^+$); 385 (85.4%, (M+1-$CH_3C_6H_4SO_3H$-CH_3CO_2H)); 367 (100%, (M+1-$CH_3C_6H_4SO_3H$-CH_3CO_2H-H_2O));

$[\alpha]^D_{295}$ -49° (c 1.0, chloroform) (lit.[208] -47°).

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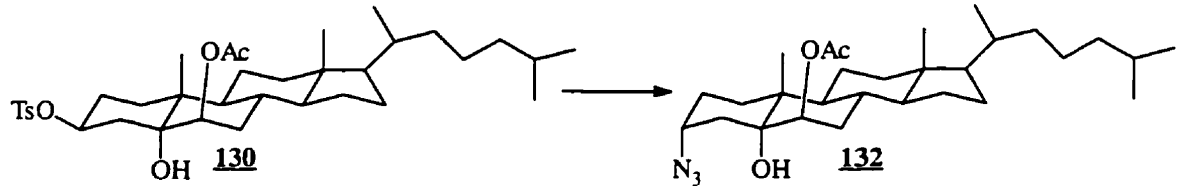
[208] Bourdon, R.; Ranisteano, S. *Bull. Soc. Chim. Fr.* **1960**, 1982

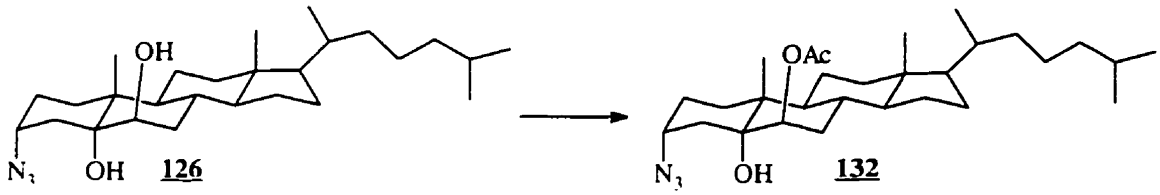
Synthesis of 6-acetyl-3α-azido-5,6β-dihydroxycholest-5α-ane 132

Method A: from 6-acetyl-3-tosyl-3β,5,6β-trihydroxycholest-5α-ane 130



To a solution of 6-acetyl-3-tosyl-3β,5,6β-trihydroxycholest-5α-ane **130** (470 mg. 0.76 mmol) in 8 ml dry DMF stirred at RT under Ar was added lithium azide (67 mg, 1.5 mg) and the mixture was warmed up to 100°C and stirred at that temperature for 1.5 h, after which time TLC indicated complete disappearance of the starting tosylate **em100**. The mixture was allowed to cool down to RT and evaporated *in vacuo* to an oily residue. This crude product was dissolved in 50 ml dichloromethane and washed twice with 10 ml distilled water. The organic layer was dried on magnesium sulfate then evaporated *in vacuo*. The crude product was recrystallized from acetone:methanol to an off-white solid (336 mg, 91%) m.p. 77°C (lit.[209] 77-78°C).

Method B: from 3α-azido-5,6β-dihydroxycholest-5α-ane 126



To a solution of 3α-azido-5,6β-dihydroxycholest-5α-ane **126** (1.02 g, 2.29 mmol) in 15 ml dry pyridine stirred at 0°C under Ar was added 4-dimethylaminopyridine (280 mg, 2.29 mmol) and acetic anhydride (264 µl, 2.75 mmol). The mixture was stirred for 6h at 0°C until TLC indicated a complete reaction. The mixture was then evaporated *in vacuo* to a yellow oily residue, then dissolved in 250 ml of dichloromethane and washed twice with 30 ml of a 10% solution of ammonium chloride, then with 30 ml of brine. The organic

[209] Wittiak, D.T.; Parker, R.A.; Dempsey, M.E.; Ritter, M.C. *J. Med. Chem.* **1971**, *14*, 684

layer was then dried on magnesium sulfate and evaporated to an off-white solid. The crude compound could be recrystallized from acetone:methanol to give 1.04 g of an off-white solid (93.1%), m.p. 76-77°C.

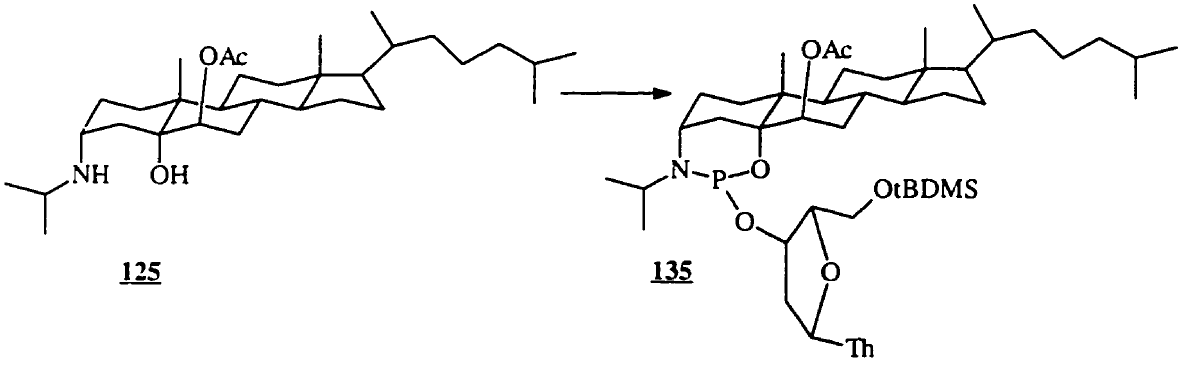
^{1}H NMR (270 MHz, $CDCl_3$) δ 4.71 (m, 1H, **H_6**); 4.03 (m, 1H, **H_3**); 3.60 (b, **OH**); 2.04 (s, 3H, CH_3C=O); 2.01-0.67 (several multiplets, **H_1**, **H_2**, **H_4**, **H_7-H_{27}**);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 170.15 (**C**=O); 75.90 (**C_6**); 73.55 (**C_5**); 58.16 (**C_3**); 56.21, 55.81, 44.87, 42.78, 39.97, 39.58, 39.19, 36.22, 35.90, 33.63, 30.92, 30.70, 28.29, 20.07, 24.96, 24.13, 23.94, 22.89, 22.63, 21.57, 20.69, 18.72, 16.38, 12.26 (**C_1**, **C_2**, **C_4**, **C_7-C_{27}**);

MS (FAB, NBA + NaCl) m/z 488 (10%, $(M+H)^+$); 460 (53.6%, $(M+1-N_2)$); 442 (63.4%, $(M+1-N_2-H_2O)$); 400 (64.1%, $(M+1-N_2-CH_3CO_2H)$); 382 (100%, $(M+1-N_2-H_2O-CH_3CO_2H)$);

$[\alpha]^D_{295}$ -57.9° (c 0.95, ethyl acetate).

Synthesis of cholestane-derived oxazaphosphorinane <u>135</u>



<u>125</u> <u>135</u>

To a solution of phosphorus trichloride (87 µl, 1 mmol) in 5 ml dry chloroform stirred at 0°C under Ar was added a mixture of 6β-acetoxy-5-hydroxy-3α–N-isopropylamino-cholest-5α-ane <u>125</u> (503 mg, 1 mmol) and triethylamine (307 µl, 2.2 mmol) in 5 ml dry chloroform over 1h. At the end of the addition, ^{31}P NMR indicated the presence of a single signal at δ 153.9 ppm, corresponding to the intermediate chlorophosphoramidite <u>134</u>. The mixture was warmed up to 60°C and a mixture of 5'-O-tBDMS-thymidine (356 mg, 1 mmol) and triethylamine (153 µl, 1.1 mmol) in 5 ml dry chloroform was added over 5 min. The mixture was allowed to cool down to RT and ^{31}P

NMR then indicated a single signal at δ 129.2 ppm. This crude mixture was concentrated *in vacuo* to a thick oil, then poured into 30 ml of ethyl acetate (previously washed twice with a saturated solution of sodium bicarbonate then dried on magnesium sulfate) to precipitate triethylammonium chloride. The salt was filtered and the filtrate was evaporated *in vacuo* to a colorless oil which was purified by flash chromatography, using hexanes:ethyl acetate:triethylamine 75:12:13 to 70:15:15. The product (R_f = 0.37 in hexanes:ethyl acetate:triethylamine 70:15:15) was collected as a white solid in 70% yield (614 mg), m.p. 91-93°C.

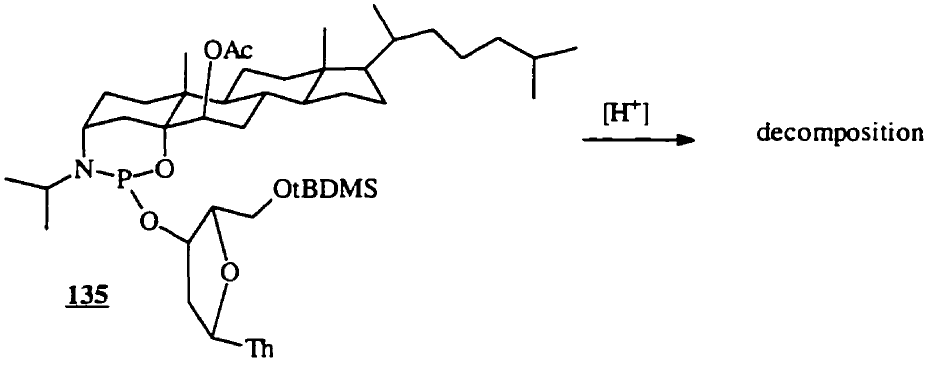
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, 1H, N-C**H**=C, $^4J_{H-H}$ = 1.0 Hz); 6.35 (dd, 1H, $\mathbf{H_{1'}}$, $^3J_{1'-2'a}$ = 6.8 Hz ; $^3J_{1'-2'b}$ = 6.0 Hz); 4.79 (m, 1H, $\mathbf{H_{6\alpha}}$); 4.61 (m, 1H, $\mathbf{H_{3'}}$); 4.05 (m, X of ABX, 1H, $\mathbf{H_{4'}}$); 3.88 (B of ABX, 1H, $\mathbf{H_{5'}b}$, $^2J_{5'b-5'a}$ = 11.5 Hz, $^3J_{5'b-4'}$ = 2.2 Hz); 3.78 (A of ABX, $\mathbf{H_{5'a}}$, $^2J_{5'a-5'b}$ = 11.5 Hz, $^3J_{5'a-4'}$ = 2.0 Hz); 3.28 (m, 1H, $\mathbf{H_{3\beta}}$); 3.18 (h, 1H, NC**H**$(CH_3)_2$, $^3J_{H-H}$ = 6.8 Hz); 2.43 (ddd, 1H, $\mathbf{H_{2'b}}$, $^2J_{2'b-2'a}$ = 13.5 Hz, $^3J_{2'b-1'}$ = 6.0 Hz, $^3J_{2'b-3'}$ = 3.0 Hz); 2.28 (m, 1H, $\mathbf{H_{4\beta}}$); 2.16 (ddd, 1H, $\mathbf{H_{2'a}}$, $^2J_{2'a-2'b}$ = 13.5 Hz, $^3J_{2'a-1'}$ = 6.8 Hz, $^3J_{2'a-3'}$ = 7.0 Hz); 2.03 (m, 1H, $\mathbf{H_{4\alpha}}$) ; 2.01 (s, 3H, C**H**$_3$COO); 1.90 (d, 3H, CH=CC**H**$_3$, $^4J_{H-H}$ = 1.0 Hz); 1.82-0.64 (37H, **cholestane backbone**); 1.19 (d, 6H, NCH(C**H**$_3)_2$, $^3J_{H-H}$ = 6.8 Hz); 0.91 (s, 9H, SiC(C**H**$_3)_3$); 0.097, 0.093 (2s, 6H, Si(C**H**$_3)_2$);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.79 (s, CH_3COOC_6); 163.51 (s, NH**C**=OC(CH_3)); 149.92 (s, N**C**=ONH); 135.72 (s, N**C**=C(CH_3)); 110.43 (s, NC=**C**(CH_3)); 86.75 (d, $\mathbf{C_{4'}}$, $^3JC_{4'-P}$ = 5.5 Hz); 85.03 (s, $\mathbf{C_{1'}}$); 78.34 (d, $\mathbf{C_5}$, $^2J_{C5-P}$ = 8.2 Hz); 74.87 (s, $\mathbf{C_6}$); 72.22 (d, $\mathbf{C_{3'}}$, $^2J_{C3'-P}$ = 20.1 Hz); 62.90 (s, $\mathbf{C_{5'}}$); 49.10 (d, N**C**H$(CH_3)_2$, $^2J_{C-P}$ = 27.5 Hz); 47.67 (d, $\mathbf{C_3}$, $^2J_{C3-P}$ = 5.5 Hz); 56.03; 55.62; 48.99; 45.96; 44.86; 42.47; 39.68 (d, J = 2.7); 39.55 (d, J = 2.7); 39.30; 35.98; 35.66; 31.22; 30.16; 29.94; 28.03; 27.81; 25.80; 23.97; 23.92; 23.88; 23.71; 22.62; 22.37; 21.84 (d, J = 10.1); 21.19; 20.42; 18.48; 14.53; 12.33; 11.94; 11.30 ($\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_4}$, $\mathbf{C_7}$-$\mathbf{C_{27}}$, CH_3C=O, NCH$(CH_3)_2$, SiC$(CH_3)_3$, $\mathbf{C_{2'}}$, C=C(CH_3)) -5.46, -5.57 (Si$(CH_3)_2$);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 129.2;

MS (FAB, NBA) m/z 888 (3.0%, $(M+H)^+$); 762 (5.3%, $(M-C_5H_5N_2O_2)$); 339 (100.0%, (5'-tBDMS-thymid-3'-yl, $C_{16}H_{27}N_2O_4Si$)); 367 (46.1%, $(M+1-C_{27}H_{42})$).

Attempts to displace the amine moiety of oxazaphosphorinane 135



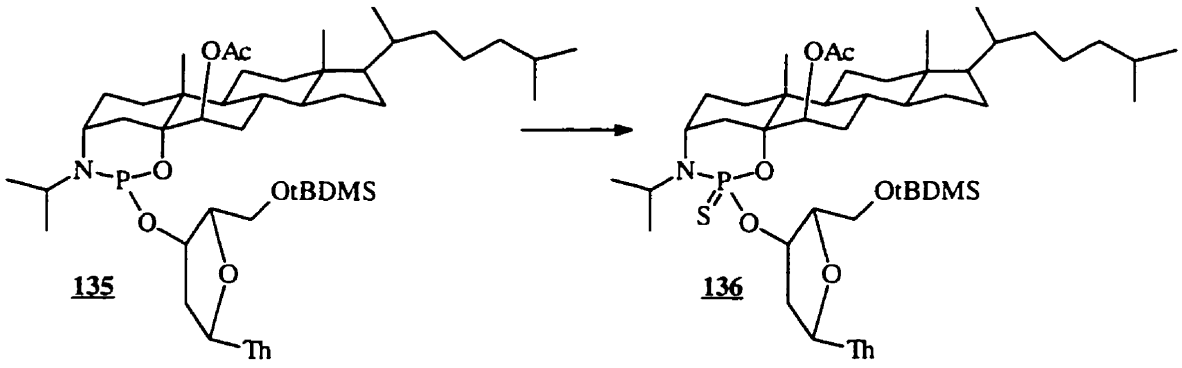
Representative procedure

Into a dry NMR tube was introduced oxazaphosphorinane **135** (44 mg, 0.05 mmol). The tube was equipped with a septum and kept in vacuo overnight, then it was flushed with Ar. Dry chloroform (0.3 ml) was introduced, and the tube was cooled down to 0°C. A solution of 2-bromo-4,5-dicyanoimidazole (22 mg, 0.11 mmol) and 3'-O-tBDPS-thymidine (24 mg, 0.05 mmol) in dry chloroform (0.3 ml), pre-cooled down to 0°C, was then introduced. The tube was shaken, and 31P NMR was then recorded. The precursor started disappearing immediately, giving rise to one phosphite triester at 141.8 ppm, as well as to several decomposition products between 0 and 15 ppm. After 15 min., the signal corresponding to 135 had completely disappeared, giving rise to the same products. The phosphite triester then accounted for about 30% of the total. However, after 30 min. the latter had completely disappeared.

The results were similar when no 3'-O-tBDPS-thymidine was introduced. It was also the case when the reaction was performed on 400 mg of oxazaphosphorinane **135**. Chromatographic separation of the many components of the mixture could not be achieved, and NMR as well as MS analysis of the mixture after chromatography did not lead to any conclusive structure.

The results were consistent with the previous ones when **143**, lacking the 6-acetoxy group, was tested.

Synthesis of 2-thiooxazaphosphorinane 136



To a solution of oxazaphosphorinane **135** (50 mg, 0.056 mmol) in 1 ml dry THF stirred at RT under Ar was added Beaucage's reagent (12 mg, 0.060 mmol). After 5 min. reaction, ^{31}P NMR showed the complete disappearance of the peak corresponding to oxazaphosphorinane **135** at 129.2 ppm, and the appearance of a single peak at 65.9 ppm, corresponding to the thio derivative **136**. The solvent was evaporated, and the compound purified by column chromatography, using ethyl acetate:hexanes:triethylamine 10:80:10 as an eluent to yield 48 mg of pure **136** in 93.3% yield.

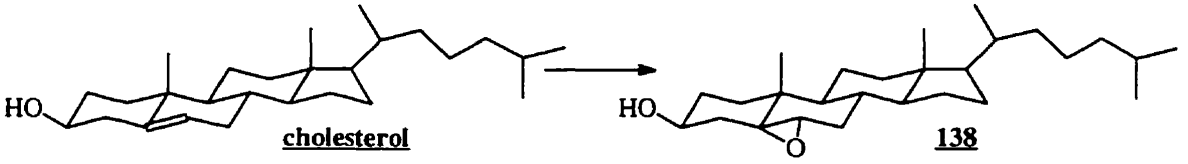
^1H NMR (500 MHz, $CDCl_3$) δ 7.53 (s, 1H, C=C**H**); 6.35 (dd, 1H, $\mathbf{H_{1'}}$, $^3J_{1'-2'a}$ = 9.5 Hz, $^3J_{1'-2'b}$ = 5.4 Hz); 4.94 (dd, 1H, $\mathbf{H_3}$', $^3J_{3'-2'a}$ = 11.5 Hz, $^3J_{3'-4'}$ = 5.5 Hz); 4.80 (m, 1H, $\mathbf{H_6}$); 4.37 (m, 1H, $\mathbf{H_{4'}}$); 3.94-4.02 (m, 2H, NC**H**$(CH_3)_2$, $\mathbf{H_{5'b}}$); 3.86 (A of ABX, 1H, $\mathbf{H_{5'a}}$, $^3J_{3'a-4'}$ = 1.5 Hz, $^2J_{5'a-5'b}$ = 11.0 Hz); 3.53 (m, $\mathbf{H_3}$); 2.47 (B of ABX, 1H, $\mathbf{H_{2'b}}$, $^2J_{2'b-2'a}$ = 13.4 Hz, $^3J_{2'b-1'}$ = 5.4 Hz); 2.02 (s, 3H, $C\mathbf{H_3}CO_2$); 1.87 (s, 3H, C=CC**H**$_3$); 2.20-0.63 (several multiplets, 49H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_7}$-$\mathbf{H_{27}}$, NCH$(C\mathbf{H_3})_2$); 0.90 (s, 9H, SiC$(C\mathbf{H_3})_3$); 0.12, 0.11 (2s, 6H, Si$(C\mathbf{H_3})_2$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 169.80 (CH_3**C**=OO); 163.58 (NH**C**=O**C**(CH_3)); 150.13 (N**C**=ONH); 135.37 (N**C**=C(CH_3); 110.91 (NC=**C**(CH3)); 87.15 (d, $\mathbf{C_{4'}}$, $^3J_{C4'-P}$ = 12.1 Hz); 84.82 ($\mathbf{C_{1'}}$); 78.33 (d, $\mathbf{C_5}$, $^2J_{C5-P}$ = 6.5 Hz); 77.20 ($\mathbf{C_{3'}}$, overlap with $CDCl_3$); 73.82 ($\mathbf{C_6}$); 63.76 ($\mathbf{C_{5'}}$); 47.76 (d, NCH$(CH_3)_2$, $^2J_{C-P}$ = 6.3 Hz); 47.33 ($\mathbf{C_3}$); 55.94, 55.21, 45.86, 44.92, 42.52, 40.18 (d, J = 3.0 Hz), 40.08, 39.42, 39.35, 36.06, 35.75, 31.21, 30.35, 30.09, 29.63, 29.07, 28.11, 27.93, 25.93, 23.99, 23.77, 22.75, 22.50, 22.28 (d, J = 8.6 Hz), 21.24, 20.47, 19.85, 14.96, 12.43, 12.03, 10.26 ($\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_4}$, $\mathbf{C_7}$-$\mathbf{C_{27}}$, CH_3**C**=OO, NCH$(C\mathbf{H_3})_2$, $\mathbf{C_{2'}}$, C=**C**(CH_3), Si**C**$(CH_3)_3$); -5.35, -5.43 (Si$(C\mathbf{H_3})_2$;

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 65.9;

MS (FAB, NBA) m/z 920 (3.4%, $(M+H)^+$); 794 (2.1%, (M-thymine)); 582 (15.2%, (M+1-(5'-O-tBDMS-thymid-3-yl)); 339 (100.0%, (5'-O-tBDMS-thymidine+1-H_2O)).

Synthesis of 3β-hydroxycholesteryl 5,6-α-oxide <u>138</u>



<u>cholesterol</u> <u>138</u>

To a solution of freshly recrystallized cholesterol (387 mg, 1.0 mmol) in 10 ml dry dichloromethane kept at 25°C was added a solution of m-chloroperbenzoic acid (377 mg of a 55% sample, 1.2 mmol) in 5 ml dry dichloromethane, over a period of time of 1h. After 3 h, TLC indicated the reaction had gone to completion, and the excess peracid was destroyed by addition of a 5% solution of sodium thiosulfate. The organic layer was washed once more with the same sodium thiosulfate solution, twice with a saturated solution of sodium bicarbonate, then once with brine. It was dried over magnesium sulfate and evaporated to a white solid. It was recrystallized twice from aqueous acetone and gave the α- isomer in 84.1% yield, m.p. 140-142°C (lit.[210] 141-143°C).

^1H NMR (200 MHz, $CDCl_3$) δ 5.1 (b, 1H, **OH**); 3.93 (m, 1H, $\mathbf{H_3}$); 2.90 (m, 1H, $\mathbf{H_6}$); 2.20-0.60 (m, 43H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_7}$-$\mathbf{H_{27}}$);

MS (EI) m/z 402 (61.2%, $M^{+\bullet}$); 384 (35.1%, ($M-H_2O$));

$[\alpha]^{D}_{295}$ -45.2° (c 1.5, $CHCl_3$) (lit.[210] -43°)

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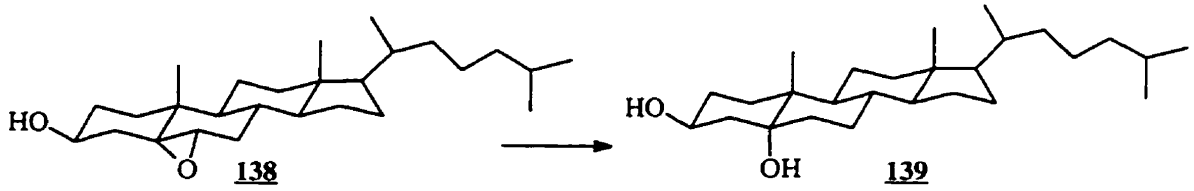
[210] Fieser, L. F.; Fieser, M. "*Reagents for Organic Synthesis, vol. 1*" **1967**, John Wiley and Sons Eds., p. 136

Synthesis of 3β,5-dihydroxycholest-5α-ane 139



To a solution of α-cholesteryl-5,6-oxide **138** (404 mg, 1.0 mmol) in 10 ml dry diethyl ether was added cautiously $LiAlH_4$ (2.0 mmol, 70 mg). The mixture was refluxed for 15h, then worked up with water and a solution of sodium hydroxide as indicated by the method reported by Fieser and Fieser[211] . After filtration of the precipitate, the ether layer was washed once with 1 ml brine, dried over magnesium sulfate and evaporated to 377 mg of a white solid (93.2 % yield). m.p. 223-225°C (lit.[212] 225°C).

^{1}H NMR (200 MHz, $CDCl_3$) δ 3.87 (m, 1H, $\mathbf{H_3}$); 2.09-0.72 (several multiplets, 45H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_6}$-$\mathbf{H_{27}}$);

^{13}C NMR[213] (75.3 MHz, $CDCl_3$) δ 74.41 ($\mathbf{C_5}$); 67.12 ($\mathbf{C_3}$); 56.73, 56.61 ($\mathbf{C_{14}}$, $\mathbf{C_{17}}$); 46.06, 45.70 ($\mathbf{C_4}$, $\mathbf{C_9}$); 43.32 ($\mathbf{C_{13}}$); 40.86 ($\mathbf{C_{12}}$); 40.04 ($\mathbf{C_{24}}$); 39.57 ($\mathbf{C_{10}}$); 36.75 ($\mathbf{C_{22}}$); 36.35 ($\mathbf{C_{20}}$); 35.61 ($\mathbf{C_8}$); 35.47 ($\mathbf{C_6}$); 32.54, 31.84 ($\mathbf{C_1}$, $\mathbf{C_2}$); 28.87 ($\mathbf{C_{16}}$); 28.57 ($\mathbf{C_{25}}$); 26.71 ($\mathbf{C_7}$); 24.84 ($\mathbf{C_{15}}$); 24.42 ($\mathbf{C_{23}}$); 22.05 ($\mathbf{C_{11}}$); 23.12 ($\mathbf{C_{26}}$, $\mathbf{C_{27}}$); 19.25 ($\mathbf{C_{21}}$); 16.48 ($\mathbf{C_{19}}$); 12.68 ($\mathbf{C_{18}}$);

M.S. (EI) 404 (1.3%, $M^{+\bullet}$); 386 (100.0%, ($M-H_2O$)); 368 (35.7%, ($M-2H_2O$)).

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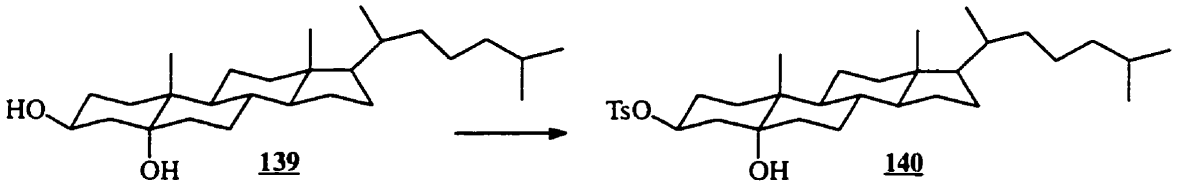
[211] Fieser, L. F.; Fieser, M. "*Reagents for Organic Synthesis, vol. 1*" **1967**, John Wiley and Sons Eds. p. 584

[212] Plattner, P. A.; Petrzilka, T.; Lang, W. *Helv. Chim. Acta* **1944**, *27*, 513

[213] assignments taken from Konno, C.; Hikino, H. *Tetrahedron*, **1976**, *32*, 325

Synthesis of 3-(*p*-toluenesulfonyl)-3β,5-dihydroxycholest-5α–ane 140

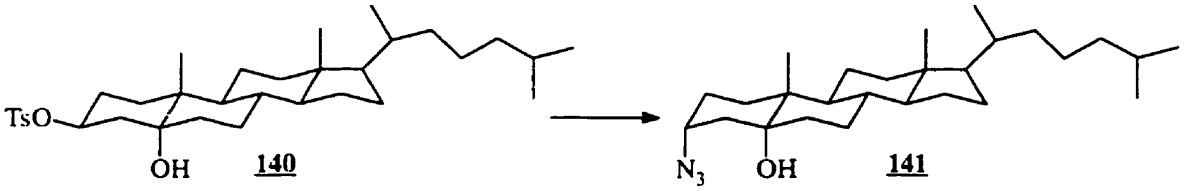


To a solution of 3β,5-dihydroxycholest-5α-ane **139** (472 mg, 1.17 mmol) in 5 ml dry pyridine stirred at 0°C under Ar, was added freshly recrystallized *p*-toluenesulfonyl chloride (245 mg, 1.29 mmol). The mixture was stirred at 0°C for another 1h and then at RT for 36h. The solvent was evaporated *in vacuo*, taken up in 5 ml diethyl ether and washed twice with 1 ml of a 10% solution of ammonium chloride, then once with 1 ml brine. The organic layer was then dried over magnesium sulfate and evaporated *in vacuo* to a sticky solid. This crude product was purified by column chromatography, using ethyl acetate:hexanes 10:90 as an eluent. The product was obtained as a white solid in 83.1% yield (542 mg).

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.76 (d, 2H, $\mathbf{H_{ortho}}$, $^3J_{ortho\text{-}meta}$ = 7.9 Hz); 7.26 (d, 2H, $\mathbf{H_{meta}}$, $^3J_{meta\text{-}ortho}$ = 7.9 Hz); 4.81 (m, 1H, $\mathbf{H_3}$); 2.46 (s, 3H, *p*-$\mathbf{CH_3}$); 2.11-0.67 (several multiplets, 45 H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_6}$-$\mathbf{H_{27}}$);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 143.25, 134.15, 129.73, 127.67 (aromatic **C**); 80.14 ($\mathbf{C_3}$); 75.36 ($\mathbf{C_5}$); 56.16, 56.04, 45.65, 42.64, 40.96, 39.99, 39.48, 38.53, 36.12, 35.77, 34.60, 34.25, 30.63, 29.21, 28.00, 27.69, 25.81, 24.06, 23.83, 22.82, 22.55, 21.66, 21.23, 18.62, 16.02, 12.11 ($\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_4}$, $\mathbf{C_6}$-$\mathbf{C_{27}}$, p-CH_3);

Synthesis of 3α-azido-5-hydroxycholest-5α-ane 141



A solution of 3-tosyl-3β,5-dihydroxychoest-5α-ane **140** (558 mg, 1.0 mmol) and lithium azide (96 mg, 2.0 mmol) in 10 ml dry DMF was stirred under Ar at 100°C for 1.5

h. TLC then indicated the reaction had gone to completion. The solvent was removed *in vacuo* and the residue was dissolved in 10 ml diethyl ether. The organic layer was then washed twice with 2 ml brine, dried over magnesium sulfate and evaporated *in vacuo* to 417 mg (97.2%) of a white solid, m.p.95-96°C;

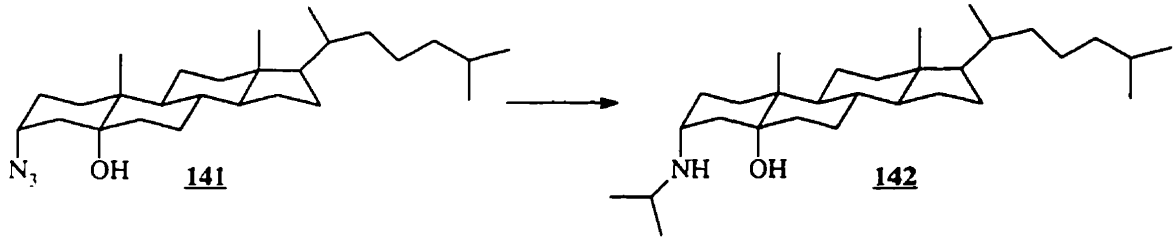
^{1}H NMR (200 MHz, $CDCl_3$) δ 3.53 (m, 1H, **H_3**); 3.02 (b, 1H, **OH**); 2.05-0.71 (several multiplets, 45H, **H_1**, **H_2**, **H_4**, **H_6-H_{27}**);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 73.15 (**C_5**); 57.91 (**C_3**); 56.21, 56.08, 45.12, 42.69, 40.03, 39.52, 39.43, 36.88, 36.17, 35.87, 34.86, 33.87, 28.29, 28.02, 26.65, 25.48, 25.16, 24.06, 23.91, 22.86, 22.59, 20.92, 18.66, 16.07, 12.16 (**C_1**, **C_2**, **C_4**, **C_6-C_{27}**);

MS (CI) m/z 430 (0.3%, $(M+H)^+$); 411 (9.4%, $(M-H_2O)$); 384 (100.0%, $(M-H_2O-N_2)$); 368 (17.1%, $(M-H_2O-HN_3)$);

$[\alpha]^D_{298}$ -2.8° (c 0.40, EtOAc).

Synthesis of 5-hydroxy-3β-isopropylaminocholest-5α-ane <u>142</u>



A solution of 3β-azido-5-hydroxycholest-5α-ane **<u>141</u>** (429 mg, 1.0 mmol) in 20 ml ethyl acetate containing a 10% mixture of Pd/C (100 mg) was shaken for 15 h under a pressure of hydrogen of 45 psi. The mixture was then filtered over a short compact pad of celite and evaporated *in vacuo* to a yellow oil. This crude intermediate primary amine was left *in vacuo* overnight. It was then dissolved in 10 ml dry methanol, and the pH of the solution was adjusted to 5.5 by addition of glacial acetic acid. Acetone (220 µl, 3.0 mmol) was added to the mixture, followed 30 min. later by sodium cyanoborohydride (95 mg, 1.5 mmol). The pH was kept at 5.5 and the mixture was stirred at RT under Ar for 20h. A saturated solution of sodium bicarbonate (2 ml) was then added, and the mixture was concentrated *in vacuo*. The crude compound was then purified by flash chromatography, to yield 317 mg (71.2%) of a white solid, m.p. 100-101°C.

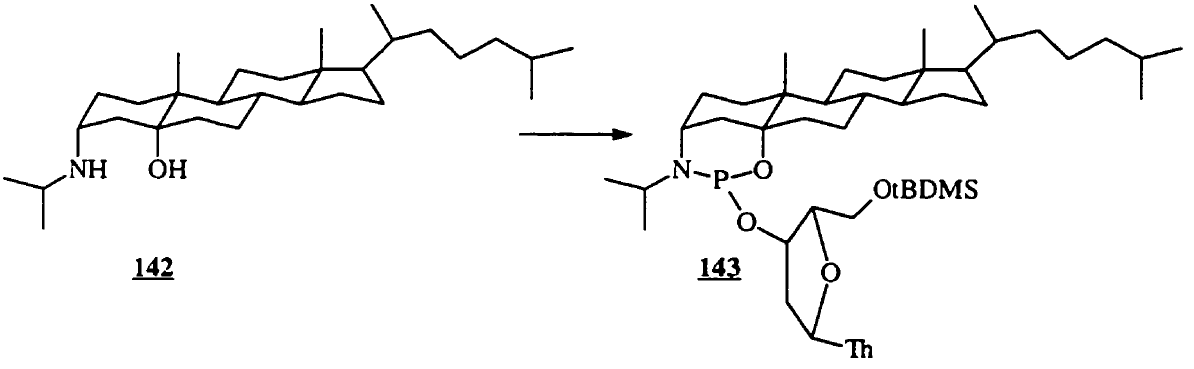
^{1}H NMR (500 MHz, $CDCl_3$) δ 3.15 (m, 1H, **H_3**); 2.94 (h, 1H, NC**H**$(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.5 Hz); 2.00-0.79 (m, 51H, **H_1**, **H_2**, **H_4**, **H_6-H_{27}**, NCH(CH_3)$_2$);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 73.70 (**C_5**); 56.13, 55.92, 48.98, 45.26, 44.75, 42.52, 39.91, 39.76, 39.35, 36.03, 35.74, 35.31, 34.85, 34.27, 28.17, 27.83, 27.44, 27.18, 25.54, 23.95, 23.82, 23.32, 22.67, 22.42, 22.40, 22.21, 20.79, 18.49, 11.962 (**C_1-C_4**, **C_6-$C_2$7**, NCH(CH_3)$_2$);

MS (CI) m/z 446 (100.0%, $(M+H)^+$); 430 (23.5%, $(M-CH_3)$); 412 (17.1%, $(M-CH_3-H_2O)$);

$[\alpha]^D_{298}$ -4.7° (c 1.6, $CHCl_3$).

Synthesis of oxazaphosphorinane <u>143</u>



<u>142</u> **<u>143</u>**

To a solution of phosphorus trichloride (12 µl, 0.13 mmol) in 1 ml dry chloroform stirred at 0°C under Ar, was added a solution of 5-hydroxy-3α-(N-isopropylamino)cholest-5α-ane **<u>142</u>** (58 mg, 0.13 mmol) and dry triethylamine (45 µl, 0.32 mmol) in 1 ml dry chloroform, over 15 min. The solution was warmed up to 50°C, then a solution of 5'-O-tBDMS-thymidine (46 mg, 0.13 mmol) and dry triethylamine (22 µl, 0.16 mmol) in 1 ml dry chloroform was added at the same temperature. The mixture was then allowed to cool down to RT, stirred for 1h at RT, then ^{31}P NMR indicated the presence of a single signal at 130.6 ppm. The solvent was evaporated *in vacuo*, then the salt was precipitated in ethyl acetate (pre-washed twice with a saturated solution of sodium bicarbonate and dried on magnesium sulfate), filtered, and the filtrate was evaporated *in vacuo*. The product was then purified by flash chromatography (hexanes:ethyl

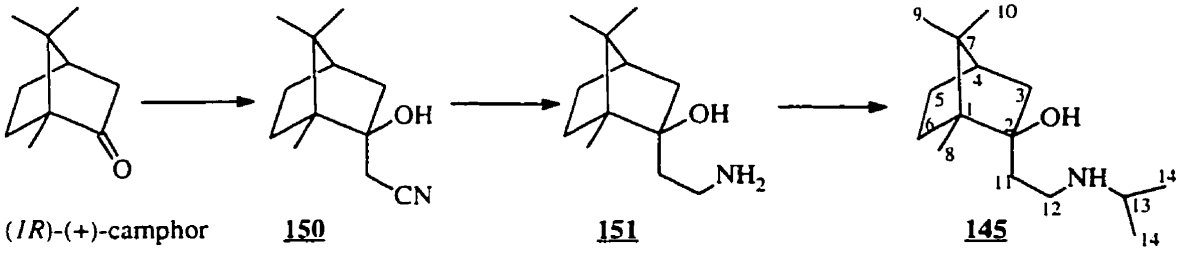
acetate:triethylamine 85:5:10), to yield the desired oxazaphosphorinane **143** as a white solid in 77.3 % yield (83 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, 1H, NC**H**=C(CH3), $^4J_{H\text{-}H}$ = 1.1 Hz); 6.33 (dd, 1H, $\mathbf{H_{1'}}$, $^3J_{1'\text{-}2'b}$ = 5.9 Hz, $^3J_{1'\text{-}2'a}$ = 7.8 Hz); 4.64 (m, 1H, $\mathbf{H_{3'}}$); 4.03 (m, 1H, $\mathbf{H_{4'}}$); 3.89 (B of ABX, 1H, $\mathbf{H_{5'b}}$, $^2J_{5'b\text{-}5'a}$ = 11.5 Hz, $^3J_{5'b\text{-}4'}$ = 2.2 Hz); 3.79 (A of ABX, 1H, $\mathbf{H_{5'a}}$, $^2J_{5'a\text{-}5'b}$ = 11.5 Hz, $^3J_{5'a\text{-}4'}$ = 2.2 Hz); 3.25 (m, 1H, $\mathbf{H_3}$); 3.19 (dh, 1H, NC**H**$(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.8 Hz, $^3J_{H\text{-}P}$ = 6.3 Hz); 2.31 (ddd, 1H, $\mathbf{H_{2'b}}$, $^2J_{2'b\text{-}2'a}$ = 13.5 Hz, $^3J_{2'b\text{-}1'}$ = 5.9 Hz, $^3J_{2'b\text{-}3'}$ = 2.4 Hz); 2.02 (ddd, 1h, $\mathbf{H_{2'a}}$, $^2J_{2'a\text{-}2'b}$ = 13.5 Hz, $^3J_{2'a\text{-}1'}$ = 7.8 Hz, $^3J_{2'a\text{-}3'}$ = 6.3 Hz); 1.90 (d, 3H, d, CH=C($\mathbf{CH_3}$), $^4J_{H\text{-}H}$ = 1.1 Hz); 2.25-0.62 (several multiplets, 60H, $\mathbf{H_1}$, $\mathbf{H_2}$, $\mathbf{H_4}$, $\mathbf{H_6}$-$\mathbf{H_{27}}$, NCH($\mathbf{CH_3}$)$_2$, SiC($\mathbf{CH_3}$)$_3$); 0.11 (s, 6H, Si($\mathbf{CH_3}$)$_2$);

^{13}C NMR (67.94 MHz, $CDCl_3$) δ 163.75 (NH**C**=OC(CH_3)); 150.28 (**NC**=ONH); 135.52 (NCH=**C**(CH_3)); 110.83 (NCH=**C**(CH_3)); 87.13 (d, $\mathbf{C_{4'}}$, $^3J_{C4'\text{-}P}$ = 6.7 Hz); 84.87 ($\mathbf{C_{1'}}$); 79.63 (d, $\mathbf{C_5}$, $^2J_{C5\text{-}P}$ = 8.8 Hz); 72.74 (d, $\mathbf{C_{3'}}$, $^2J_{C3'\text{-}P}$ = 23.3 Hz); 63.26 ($\mathbf{C_{5'}}$); 56.25 (d, NCH(CH_3)$_2$, $^2J_{C\text{-}P}$ = 18.6 Hz); 49.28 (d, $\mathbf{C_3}$, $^2J_{C3\text{-}P}$ = 26.9 Hz); 48.23, 46.24, 45.51, 42.71, 40.26, 40.09, 39.57, 36.25, 35.90, 34.74, 34.46, 34.22, 28.56, 28.31, 28.03, 26.04, 24.24.20, 24.17, 23.96, 22.84, 22.59, 22.16, 22.12, 20.91, 18.73, 18.44, 14.73, 12.54, 12.13, 11.45 ($\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_4}$, $\mathbf{C_6}$-$\mathbf{C_{27}}$, NCH($\mathbf{CH_3}$)$_2$, SiC($\mathbf{CH_3}$)$_2$, CH=C($\mathbf{CH_3}$)); -5.27, -5.33 (Si($\mathbf{CH_3}$)$_2$);

MS (FAB, NBA) m/z 830 (4.6%, $(M+H)^+$); 369 (22.9%, (cholestadiene+1)); 339 (100.0%, (5'-O-tBDMS-thymidine+1-H2O)).

Synthesis of (*1R,2S,4R*)-2-(2-(N-isopropyl)amino)ethyl-2-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptane 145



(*1R*)-(+)-camphor **150** **151** **145**

To a solution of dry acetonitrile (210 µl, 4.0 mmol) in 40 ml dry THF stirred at -78°C under Ar was added a 1.6 M solution of butyllithium in hexanes (2.75 ml, 4.4 mmol) over 30 min. The mixture was stirred for 15 min at -78°C then 30 min. at -40°C, then cooled down to -78°C. At that temperature was added a solution of freshly sublimed (*1R*)-(+)-camphor (533 mg, 3.5 mmol) in 2 ml dry THF over 15 min. The mixture was stirred for 2h at -78°C then 6h at 0°C, until TLC showed complete consumption of camphor. An attempt was made to isolate and purify the intermediate cyanoalcohol **150**, however this one decomposed before it could be purified. However it gave a satisfactory MS analysis. Therefore to this crude mixture cooled down to 0°C was added $LiAlH_4$ (304 mg, 8 mmol.) and the mixture was stirred at RT under Ar for 14h, until TLC indicated complete disappearance of the intermediate nitrile. To the crude mixture cooled down to 0°C again, was added slowly 310 µl water, then 460 µl of a 2N sodium hydroxide solution, then 1.2 ml water. The white solid thus formed was filtered, washed with water, and the resulting filtrate evaporated *in vacuo* to a yellow oily residue. This crude amine **151** was not purified, but its MS analysis gave satisfactory results. After standing *in vacuo* for 12h, the mixture was dissolved in 40 ml dry methanol, then its pH was adjusted to 5.5 by slow addition of acetic acid. Acetone (1.18 ml, 16 mmol) was added to the mixture, followed 1h later by sodium cyanoborohydride. The reaction lasted 4h, during which pH was maintained around 5.5 by addition of acetic acid, after which time TLC indicated that the reaction had gone to completion. To the mixture was added a saturated solution of sodium bicarbonate (2 ml), and it was concentrated *in vacuo*. It was then taken up in 100 ml of dichloromethane, and washed three times with 10 ml of a 10% solution of sodium carbonate. The organic layer was then dried on magnesium sulfate and evaporated to a yellow sticky gum. The product was purified by flash chromatography, using dichloromethane then dichloromethane:triethylamine 98:2 as an eluent, to yield 604 mg (63.2% from camphor) of a slightly yellow sticky solid.

^{1}H NMR (500 MHz, $CDCl_3$) (assignments based on COSY and HETCOR) δ 2.95 (dq, 1H, $\mathbf{H_{12b}}$, $^2J_{12b\text{-}12a}$ = 12.2 Hz, $^3J_{12b\text{-}11b}$ = 6.8 Hz, $^3J_{12b\text{-}11a}$ = 3.4 Hz); 2.83 (qd, 1H, $\mathbf{H_{12a}}$, $^2J_{12a\text{-}12b}$ = 12.2 Hz, $^3J_{12a\text{-}11b}$ = 9.8 Hz, $^3J_{12a\text{-}11a}$ = 2.9 Hz); 2.73 (h, 1H, $\mathbf{H_{13}}$, $^3J_{13\text{-}14}$ = 6.3 Hz); 2.04 (dt, 1H, $\mathbf{H_{3b}}$, $^2J_{3b\text{-}3a}$ = 12.7 Hz, $^3J_{3b\text{-}4}$ = 3.9 Hz); 1.66 (m, 3H, $\mathbf{H_4}$ + $\mathbf{H_{5b}}$ + $\mathbf{H_{11b}}$); 1.52 (dq, 1H,

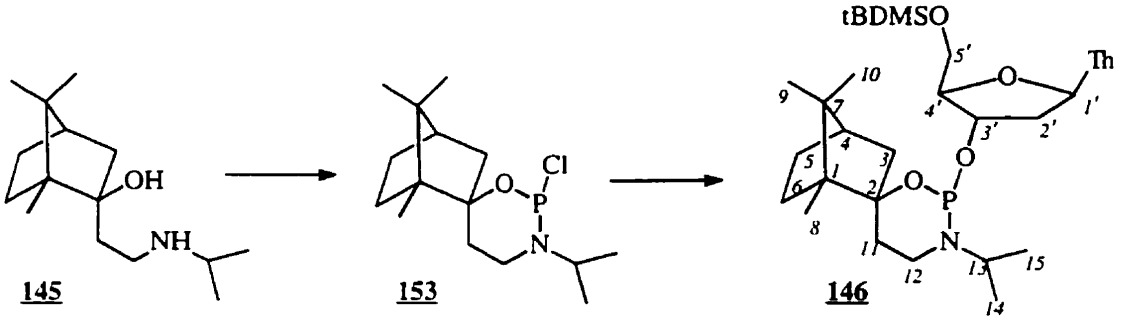
$\mathbf{H_{11a}}$, $^{2}J_{11a\text{-}11b}$ = 14.6 Hz, $^{3}J_{11a\text{-}12b}$ = 3.4 Hz, $^{3}J_{11a\text{-}12a}$ = 2.9 Hz); 1.34 (m, 3H, $\mathbf{H_{3a}}$ + $\mathbf{H_{6b}}$ + $\mathbf{H_{6a}}$); 1.04 (d, 6H, $\mathbf{H_{14}}$, $^{3}J_{14\text{-}13}$ = 6.3 Hz); 0.92 (m, 1H, $\mathbf{H_{5a}}$); 1.11, 0.87, 0.83 (3s, 3x3H, $\mathbf{H_8}$ + $\mathbf{H_9}$ + $\mathbf{H_{10}}$);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 81.77 ($\mathbf{C_2}$); 51.92 ($\mathbf{C_1}$); 49.37 ($\mathbf{C_7}$); 48.70 ($\mathbf{C_{13}}$); 47.21 ($\mathbf{C_3}$); 44.98 ($\mathbf{C_4}$); 44.12 ($\mathbf{C_{12}}$); 36.95 ($\mathbf{C_{11}}$); 30.15 ($\mathbf{C_6}$); 26.96 ($\mathbf{C_5}$); 22.78, 22.40 (2x$\mathbf{C_{14}}$); 21.34, 20.84, 11.03 ($\mathbf{C_8}$, $\mathbf{C_9}$, $\mathbf{C_{10}}$);

MS (CI) m/z 240 (100.0%, $(M+H)^+$); 224 (18.1%, $(M-CH_3)$); 222 (14.2%, $(M+1-H_2O)$);

$[\alpha]^{D}_{295}$ +30.1° (c 1.8, EtOAc).

Synthesis of camphor-derived oxazaphosphorinane <u>146</u>



To a solution of phosphorus trichloride (87 µl, 1.0 mmol) in dry chloroform (3 ml) stirred at 0°C under Ar, was added a solution of (*1R,2S,4R*)-2-(2-(N-isopropyl)amino)ethyl-2-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptane **<u>145</u>** (236 mg, 1.0 mmol) and dry triethylamine (307 µl, 2.2 mmol) in dry chloroform (3 ml), over 1h. After that time, ^{31}P NMR indicated the presence of a single signal at 161.5 ppm, corresponding to intermediate **<u>153</u>**. At the same temperature, a solution of 5'-O-tBDMS-thymidine (356 mg, 1.0 mmol) and dry triethylamine (154 µl, 1.1 mmol) in 3 ml dry chloroform was added over 1h. The mixture was stirred at RT for 1h, then ^{31}P NMR indicated the presence of two signals at 140.0 and 136.7 ppm respectively, in a ratio of 3:1. Refluxing the mixture as long as 48h did not change this ratio. The mixture was evaporated *in vacuo* and the salt was precipitated by addition of ethyl acetate (pre-washed twice with a saturated solution of sodium bicarbonate and dried over magnesium sulfate). After filtration of triethylammonium chloride, the filtrate was concentrated *in vacuo* and the mixture of

diastereomers was isolated by flash chromatography (hexanes:ethyl acetate:triethylamine 85:15:10) in 77.3% yield (482 mg) as a colorless oil. The two diastereomers could not be separated chromatographically. Analyses were performed on a 2:1 diastereomeric mixture. The ^{1}H NMR signals of the major component of the mixture are given, the signals for the minor component frequently overlapped with these of the major. When both signals are given, the indexes M and m indicate major and minor component respectively. The ^{13}C NMR signals of both components could be determined, and are also referred as M and m. Numbers refer to the camphor derivative, primed numbers to the nucleoside unit.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (M) (d, 1H, NC**H**=C(CH_3), $^{3}J_{H\text{-}H}$ = 1.2 Hz); 7.48 (m) (d, 1H, NC**H**=C(CH_3), $^{3}J_{H\text{-}H}$ = 1.2 Hz); 6.33 (M) (dd, 1H, $\mathbf{H_{1'}}$, $^{3}J_{1'\text{-}2'b}$ = 5.6 Hz, $^{3}J_{1'\text{-}2'}$ = 8.5 Hz); 4.57-4.53 (m) (m, 1H, $\mathbf{H_{3'}}$); 4.48-4.51 (M) (m, 1H, $\mathbf{H_{3'}}$); 4.08 (M) (m, 1H, $\mathbf{H_{4'}}$); 4.03 (m) (m, 1H, $\mathbf{H_{4'}}$); 3.74 (M) (B of ABX, 1H, $\mathbf{H_{5'b}}$, $^{2}J_{5'b\text{-}5'a}$ = 11.5 Hz, $^{3}J_{5'b\text{-}4'}$ = 2.1 Hz); 3.86 (m) (B of ABX, 1H, $\mathbf{H_{5'b}}$, $^{2}J_{5'b\text{-}5'a}$ = 11.5 Hz, $^{3}J_{5'b\text{-}4'}$ = 2.3 Hz); 3.75 (m) (A of ABX, 1H, $\mathbf{H_{5'a}}$, $^{2}J_{5'a\text{-}5'b}$ = 11.5 Hz, $^{3}J_{5'a\text{-}4'}$ = 2.3 Hz); 3.74 (M) (A of ABX, 1H, $\mathbf{H_{5'a}}$, $^{2}J_{5'a\text{-}5'b}$ = 11.5 Hz, $^{3}J_{5'a\text{-}4'}$ = 2.1 Hz); 3.35 (M) (dh, 1H, $\mathbf{H_{13}}$, $^{3}J_{H\text{-}H}$ = 6.6 Hz, $^{3}J_{H\text{-}P}$ = 5.9 Hz); 3.27 (M) (m, 1H, $\mathbf{H_{12b}}$); 3.08 (M) (m, 1H, $\mathbf{H_{3b}}$); 2.78 (M) (m, 1H, $\mathbf{H_{12a}}$); 2.41 (M) (dt, $\mathbf{H_{3a}}$, $^{2}J_{3b\text{-}3a}$ = 13.9 Hz, $^{3}J_{3b\text{-}4}$ = 3.7 Hz, $^{4}J_{3b\text{-}5}$ = 3.2 Hz); 2.32 (M) (B of ABX dedoubled, 1H, $\mathbf{H_{2'b}}$, $^{2}J_{2'b\text{-}2'a}$ = 12.2 Hz; $^{3}J_{2'b\text{-}1'}$ = 5.6 Hz, $^{3}J_{2'b\text{-}4'}$ = 1.5 Hz); 2.10 (m) (dt, 1H, $\mathbf{H_{3b}}$, $^{2}J_{3b\text{-}3a}$ = 13.7 Hz, $^{3}J_{3b\text{-}4}$ = 3.7 Hz, $^{4}J_{3b\text{-}5}$ = 3.4 Hz); 1.99 (M) (ddd, 1H, $\mathbf{H_{2'a}}$, $^{2}J_{2'a\text{-}2'b}$ = 12.2 Hz, $^{3}J_{2'a\text{-}1'}$ = 8.5 Hz, $^{3}J_{2'a\text{-}3'}$ = 4.4 Hz); 1.88 (M+m) (b, 3H, CH=C(C**H**$_3$)); 1.87-0.10 (several multiplets, $\mathbf{H_4}$, 2x$\mathbf{H_5}$, 2x$\mathbf{H_6}$, 3x$\mathbf{H_8}$, 3x$\mathbf{H_9}$, 3x$\mathbf{H_{10}}$, SiC(C**H**$_3$)$_3$, Si(C**H**$_3$)$_2$)

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.93 (m+M) (NH**C**=OC(CH_3); 150.33 (m+M) (N**C**=ONH); 135.49 (m) (**C**H=C(CH_3); 135.43 (**C**H=C(CH_3); 110.78 (m+M) (NCH=**C**(CH_3); 87.15 (m) (d, $\mathbf{C_{4'}}$, $^{3}J_{C4'\text{-}P}$ = 5.5 Hz); 86.70 (M) (d, $\mathbf{C_{4'}}$, $^{3}J_{C4'\text{-}P}$ = 3.7 Hz); 85.84 (m) (d, $\mathbf{C_2}$, $^{2}J_{C2\text{-}P}$ = 10.1 Hz); 85.50 (M) (d, $\mathbf{C_2}$, $^{2}J_{C2\text{-}P}$ = 10.1 Hz); 84.89 (m) ($\mathbf{C_{1'}}$); 84.86 (M) ($\mathbf{C_{1'}}$); 73.16 (M) (d, $\mathbf{C_{3'}}$, $^{2}J_{C3'\text{-}P}$ = 21.1 Hz); 72.64 (m) (d, $\mathbf{C_{3'}}$, $^{2}J_{C3'\text{-}P}$ = 20.1 Hz); 63.40 (M) ($\mathbf{C_{5'}}$); 63.30 (m) ($\mathbf{C_{5'}}$); 52.94 (M) (d, C_1, $^{3}J_{C1\text{-}P}$ = 4.6 Hz); 52.75 (m) (C_1); 40.61 (d, $C_{2'}$, $^{3}J_{C2'\text{-}P}$ = 4.6 Hz); 25.90 (SiC(C**H**$_3$)$_3$); 21.76 (d, NCH(C**H**$_3$)$_2$; $^{3}J_{C\text{-}P}$ = 10.1 Hz); 21.44 (NCH(C**H**$_3$)$_2$, overlapped with 21.38), 18.31 (SiC(C**H**$_3$)$_3$); 12.46 (CH=C(C**H**$_3$)), 49.28, 48.91, 48.64, 45.92, 45.34, 34.16, 34.14, 29.85, 26.62, 21.10,

20.93, 20.91, 10.74(C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}), -5.43, -5.50 ($Si(CH_3)_2$);

^{31}P NMR (202.3 MHz, $CDCl_3$) δ 104.1 (M); 136.8 (m);

MS (FAB, NBA) m/z 624 (5.5%, $(M+H)^+$); 339 (5'-O-tBDMS-thymidine+1-H_2O)); 163 (100.0%, (M-(5'-O-tBDMS-thymid-3'-yl-P(OH)NHiPr)));

HRMS (FAB +) $(M+H)^+$ $C_{31}H_{55}N_3O_6PSi$ (calc. 624.359779, found 624.359500).

Acid-catalyzed rearrangement of oxazaphosphorinanes 146

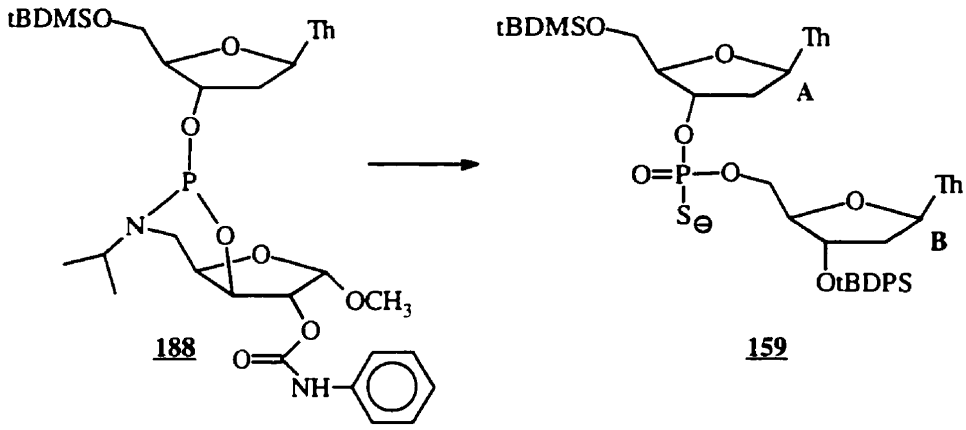
The attempts to react this mixture of oxazaphosphorinanes with or without 3'-O-tBDPS-thymidine followed the procedure indicated p.148. The decomposition products, after chromatography (ethyl acetate:triethylamine 95:5), gave very complex ^1H and ^{13}C NMR spectra, being a mixture of three compounds as suggested by ^{31}P NMR.

HRMS $(M+H)^+$ ($C_{31}H_{55}N_3O_6PSi$ (calc. 624.359779, found 624.359500).

This result absolutely correlates with the molecular weight of oxazaphosphorinane **146**, leading to the conclusion that it is a rearrangement product from **146**.

6.4. Experimentals for section 4

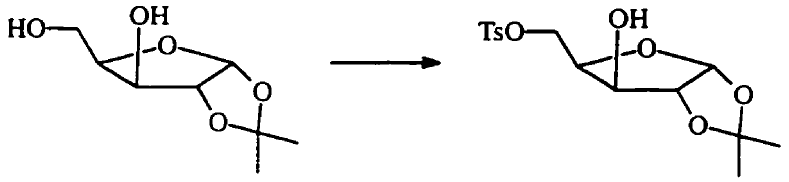
Synthesis of (5'-*ter*butyldimethylsilyl)thymid-3'-yl (3'-*ter*butyldiphenylsilylthymid-5'-yl) phosphorothioate 159



To a solution of oxazaphosphorinane **188** (50 mg, 0.07 mmol) in 0.5 ml dry acetonitrile stirred under Ar at -20°C, was added a solution of 3'-O-tBDPS-thymidine (33 mg, 0.07 mmol), 2-bromo-4,5-dicyanoimidazole (55 mg, 0.28 mmol) in 0.5 ml dry acetonitrile. Exactly 1 min. after the introduction of the second part of the mixture, Beaucage's sulfurizing reagent (14 mg, 0.07 mmol) was introduced as a solid in the reaction mixture. ^{31}P NMR revealed, after 5 min, the presence of two sets of products: one single peak at 66.9 ppm, corresponding to the intermediate phosphorothioate triester, and a second set, less intense, constituted of two peaks at 55.4 and 55.1 ppm, corresponding to the desired phosphorothioate diester, in a ratio of 5:1 respectively. Dry DMF (0.5 ml) was introduced into the reaction mixture, followed by ammonium hydroxide (0.2 ml of a 28% solution). DMF was introduced to help the reaction go to completion, as well as solubilize the final anionic species, which is very poorly soluble in acetonitrile. ^{31}P NMR then indicated the presence of only two peaks at 55.4 and 55.1 ppm, in a ratio of 28.5:1 respectively. The solvents were evaporated *in vacuo* and azeotroped twice with toluene, and the mixture was purified by silica gel chromatography, using ethyl acetate to ethyl acetate:methanol 94:6 as an eluent. Only the major diastereomer was obtained pure in 87.2% yield (57 mg) (yield calculated for the ammonium salt). The nucleosides are below referred as A and B, according to the scheme.

^{1}H NMR (500 MHz, CD_3OD) δ 7.97 (d, 1H, C**H**=C(CH3), $^4J_{H\text{-}H}$ = 1.0 Hz); 7.83 (d, 1H, C**H**=C(CH_3), $^4J_{H\text{-}H}$ = 1.0 Hz); 7.72-7.36 (m, 10H, **2xC$_6$H$_5$**); 6.46 (dd, 1H, **H$_{1'B}$**, $^3J_{1'\text{-}2'}$ = 9.3 Hz, $^3J_{1'\text{-}2'}$ = 5.4 Hz); 6.17 (dd, 1H, **H$_{1'A}$**, $^3J_{1'\text{-}2'}$ = 9.0 Hz, $^3J_{1'\text{-}2'}$ = 5.4 Hz); 4.99 (m, 1H, **H$_{3'A}$**); 4.56 (m, 1H, **H$_{3'B}$**); 4.21 (m, 1H, **H$_{4'A}$**); 4.08 (m, 1H, **H$_{4'B}$**); 3.92 (m, 1H, **H$_{5'B}$**); 3.88 (AB of ABX, 2H, **2xH$_{5'A}$**, $^2J_{5'a\text{-}5'b}$ = 11.5 Hz, $^3J_{5'b\text{-}4'}$ = 2.5 Hz, $^3J_{5'a\text{-}4'}$ = 2.0 Hz); 3.62 (m, 1H, **H$_{5'B}$**); 2.23-2.21 (m, 1H, **H$_{2'A}$**); 2.20-2.15 (m, 1H, **H$_{2'B}$**); 2.09-2.02 (m, 1H, **H$_{2'B}$**); 1.97-1.94 (m, 1H, **H$_{2'A}$**); 1.92 (d, 3H, CH=C(C**H**$_3$), $^4J_{H\text{-}H}$ = 1.0 Hz); 1.88 (d, 3H, CH=C(C**H**$_3$); $^4J_{H\text{-}H}$ = 1.0 Hz); 1.08 (s, 9H, SiC(C**H**$_3$)$_3$); 0.92 (s, 9H, SiC(C**H**$_3$)$_3$); 0.13, 0.12 (2s, 6H, Si(C**H**$_3$)$_2$).

Synthesis of 1,2-O-isopropylidene-5'-O-*p*-toluenesulfonyl-D-xylofuranose <u>178</u>



1,2-O-isopropylidene-D-xylofuranose **<u>178</u>**

To a solution of 1,2-O-isopropylidene-D-xylofuranose (47.2 mmol, 8.98 g) stirred in 500 dry pyridine under Ar at 0°C was added freshly recrystallized p-toluenesulfonyl chloride (47.2 mmol, 9.00 g) in 3 portions over 3h. The reaction was allowed to go on for another 5h at 0°C, then at RT for 8h. After concentration of the solution to a thick paste, the residue was taken up in ethyl acetate (800 ml) and washed three times with 100 ml of a 10% solution of ammonium chloride and then with 100 ml brine. The organic phase was dried over magnesium sulfate and evaporated *in vacuo* to an off-white solid. Recrystallization of this solid from dichloromethane:hexanes gave the desired compound in 92.4% yield (15.00 g) as a white solid, m.p. 132-133°C (lit.[214] 133-134°C)

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.77 (BB' of AA'BB', 2H, aromatic **H$_2$** and **H$_6$**, $^3J_{B\text{-}A}$ = 8.0 Hz); 7.33 (AA' of AA'BB', 2H, aromatic **H$_3$** and **H$_5$**, $^3J_{A\text{-}B}$ = 8.0 Hz); 5.85 (d, 1H, **H$_{1'}$**, $^3J_{1'\text{-}2'}$ = 3.5 Hz); 4.49 (d, 1H, **H$_{2'}$**, $^3J_{2'\text{-}1'}$ = 3.5 Hz); 4.33-4.25 (m, 3H, **H$_{5'a}$**, **H$_{5'b}$**, **H$_{3'}$**); 4.15

[214] Tipson, R. S. *Meth. Carbohydr. Chem.* **1963**, *2*, 246

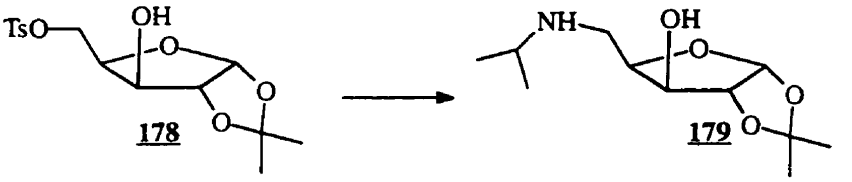
(m, 1H, $\mathbf{H_{4'}}$); 2.43 (s, 3H, $\mathbf{CH_3C_6H_4}$); 2.28 (d, 1H, **OH**, $^3J_{OH\text{-}3'}$ = 4.8 Hz); 1.45 (s, 3H, $OC(\mathbf{CH_3})_2O$); 1.28 (s, 3H, $OC(\mathbf{CH_3})_2O$);

^{13}C NMR (75.3 MHz, $CDCl_3$) δ 145.26, 132.11, 129.95, 127.94 (aromatic **C**), 112.02 ($\mathbf{O}C(CH_3)_2O$), 104.87 ($\mathbf{C_{1'}}$), 84.85 ($\mathbf{C_{2'}}$), 77.63 ($\mathbf{C_{3'}}$), 74.15 ($\mathbf{C_{4'}}$), 66.49 ($\mathbf{C_{5'}}$), 26.68, 26.11 ($OC(\mathbf{CH_3})_2O$), 21.60 ($\mathbf{CH_3C_6H_4}$);

MS (CI) m/z 345 (45.31%, $(M+H)^+$); 329 (79.3%, $(M\text{-}CH_3)$);

$[\alpha]^D_{295}$ **-15.3° (c 2.85, EtOAc).**

Synthesis of 5-deoxy-5-N-isopropylamino-1,2-O-isopropylidene-xylofuranose <u>179</u>



In a dry pressure vessel flushed with dry Ar, a solution of 1,2-O-isopropylidene-5-*p*-toluenesulfonyl-xylofuranose **<u>178</u>** (3.0 mmol, 1.032 g) in isopropylamine (20 ml) was heated at 80°C under pressure for 20 h. After that time, TLC indicated a complete reaction and the mixture was first allowed to cool down to RT and evaporated *in vacuo* to eliminate isopropylamine. The brown syrup thus obtained was then taken up in 100 ml of ethyl acetate and washed twice with a mixture of brine and 10% sodium carbonate solution (10 ml of each). The organic layer was then dried over magnesium sulfate and evaporated *in vacuo* to an amber-colored oil in a 96 % yield (0.665 g).

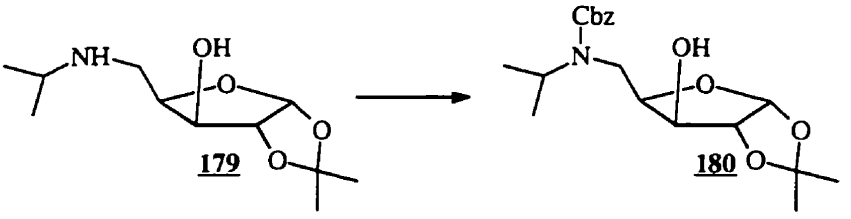
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.93 (d, 1H, $\mathbf{H_{1'}}$, $^3J_{1'\text{-}2'}$ = 3.5 Hz); 4.47 (d, 1H, $\mathbf{H_{2'}}$, $^3J_{2'\text{-}1'}$ = 3.5 Hz); 4.28 (d, 1H, $\mathbf{H_{3'}}$, $^3J_{3'\text{-}4'}$ = 3.0 Hz); 4.21 (m, 1H, $\mathbf{H_{4'}}$); 3.37 (B of ABX, 1H, $\mathbf{H_{5'b}}$, $^2J_{5'b\text{-}5'a}$ = 12.5 Hz; $^3J_{5'b\text{-}4'}$ = 3.8 Hz); 2.97 (A of ABX, 1H, $\mathbf{H_{5'a}}$, $^2J_{5'a\text{-}5'b}$ = 12.5 Hz, $^3J_{5'a\text{-}4'}$ = 1.5 Hz); 2.76 (h, 1H, N-$\mathbf{CH}(CH_3)_2$, $^3J_{H\text{-}H}$ = 6 Hz); 1.47 (s, 3H, $OC(\mathbf{CH_3})_2$); 1.31 (s, 3H, $OC(\mathbf{CH_3})_2$); 1.07 (2d, 6H, $NCH(\mathbf{CH_3})_2$, $^3J_{H\text{-}H}$ = 6 Hz);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.37 ($O\text{-}\mathbf{C}(CH_3)_2\text{-}O$); 105.06 ($\mathbf{C_{1'}}$); 86.06 ($\mathbf{C_{2'}}$); 78.19 ($\mathbf{C_{3'}}$); 76.92 ($\mathbf{C_{4'}}$); 48.70 ($\mathbf{C_{5'}}$); 45.80 ($N\mathbf{C}H(CH_3)_2$); 26.82, 26.13 ($O\text{-}\mathbf{C}(CH_3)_2\text{-}O$); 22.58, 22.26 ($NCH(\mathbf{CH_3})_2$);

MS (CI) m/z 232 (100%, $(M+H)^+$); 216 (10.2%, $(M\text{-}CH_3)$);

$[\alpha]^{D}_{295}$ +20.6° (c 2.3, EtOAc).

Synthesis of 5-deoxy-5-(N-carboxybenzyloxy-N-isopropylamino)-3-hydroxy-1,2-O-isopropylidenexylofuranose 180



To a solution of 5-deoxy-5-(N-isopropylamino-1,2-O-isopropylidene-D-xylofuranose **179** (231 mg, 1.0 mmol) and potassium bicarbonate (200 mg, 2.0 mmol) in 9 ml THF and 1 ml water stirred at RT was added benzyl chloroformate (143 µl, 1.0 mmol). After 1.5h, TLC indicated that the reaction had gone to completion. The mixture was evaporated *in vacuo*, dissolved in 30 ml ethyl acetate and washed once with 5 ml of a 10% solution of ammonium chloride and once with 5 ml brine. The organic layer was then dried over magnesium sulfate and evaporated *in vacuo* to an oil. Flash chromatography (ethyl acetate:hexanes 20:80) yielded 333 mg (91.2%) of a colorless oil that was crystallized by precipitation in cold petroleum ether, m.p. 103°C

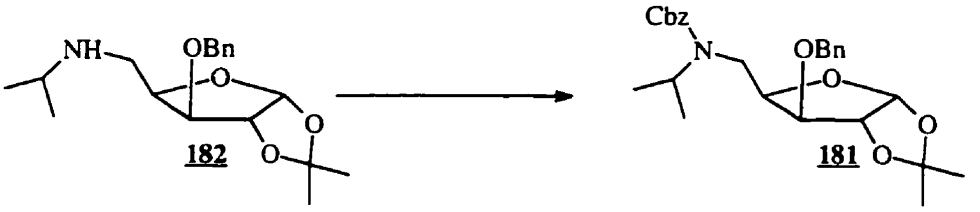
^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 7.33-7.40 (m, 5H, $C_6\mathbf{H_5}$); 5.90 (d, 1H, $\mathbf{H_1}$, $^{3}J_{1\text{-}2}$ = 3.7 Hz); 5.18 (d, 1H, $\mathbf{H_3}$, $^{3}J_{3\text{-}4}$ = 3.3 Hz); 5.16 (s, 2H, $C_6H_5C\mathbf{H_2}O$); 4.60 (d, 1H, $\mathbf{H_2}$, $^{3}J_{2\text{-}1}$ = 3.7 Hz); 4.24 (h, 1H, $NC\mathbf{H}(CH_3)_2$, $^{3}J_{H\text{-}H}$ = 7.2 Hz); 4.04 (ddd, 1H, $\mathbf{H_4}$, $^{3}J_{4\text{-}5b}$ = 10.7 Hz, $^{3}J_{4\text{-}5a}$ = 3.5 Hz, $^{3}J_{4\text{-}3}$ = 3.3 Hz); 4.02 (b, 1H, O**H**); 3.73 (B of ABX, 1H, $\mathbf{H_{5b}}$, $^{2}J_{5b\text{-}5a}$ = 15.0 Hz, $^{3}J_{5b\text{-}4}$ = 10.7 Hz); 3.20 (A of ABX, 1H, $\mathbf{H_{5a}}$, $^{2}J_{5a\text{-}5b}$ = 15.0 Hz, $^{3}J_{5a\text{-}4}$ = 3.5 Hz); 1.48, 1.32 (2s, 6H, $OC(C\mathbf{H_3})_2O$); 1.22, 1.15 (2d, 6H, $NCH(C\mathbf{H_3})_2$, $^{3}J_{H\text{-}H}$ = 7.2 Hz);

^{13}C NMR (125.7 MHz, CD_3SOCD_3, 90°C) δ 157.61(**C**=O), 128.47, 128.44, 128.11, 127.80 (aromatic **C**), 111.27 ($O\mathbf{C}(CH_3)_2O$), 104.38 ($\mathbf{C_1}$), 84.72 ($\mathbf{C_2}$), 79.94 ($\mathbf{C_4}$), 73.49 ($\mathbf{C_3}$), 67.74 ($COO\mathbf{C}H_2$), 48.40 ($\mathbf{C_5}$), 39.87 ($N\mathbf{C}H(CH_3)_2$, 26.74, 25.99 ($OC(\mathbf{C}H_3)_2O$, 20.89, 20.22 ($NCH(\mathbf{C}H_3)_2$);

MS (FAB) m/z 366 (100.0%, $(M+H)^+$); 322 (65.4%, $(M\text{-}CH_3CH{=}CH_2)$);

$[\alpha]^{D}_{295}$ +34.5° (c 1.1, ethyl acetate).

Synthesis of 3-O-benzyl-5-deoxy-5-(N-isopropyl-N-carboxybenzyloxy)amino-1,2-O-isopropylidenexylofuranose 181



To a solution of 3-O-benzyl-5-isopropylamino-5-deoxy-1,2-isopropylidene-xylofuranose **181** (10.8 mmol, 3.46 g) and potassium bicarbonate (11.0 mmol, 1.10 g) in 100 ml of a mixture of THF and water (9:1) stirred at RT was added benzyl chloroformate (10.8 mmol, 1.54 ml). After 1.5 h, the reaction was complete as indicated by TLC. The mixture was then evaporated *in vacuo* and taken up in 350 ml of ethyl acetate, washed twice with brine, and the organic layer was then dried over magnesium sulfate and evaporated to 4.87 of a yellow oil in an essentially quantitative yield. The compound was pure as evaluated by ^{1}H NMR.

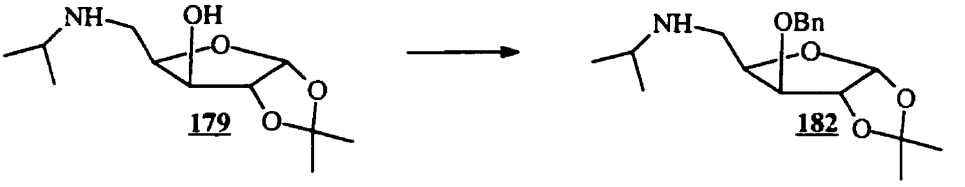
^{1}H NMR (200 MHz, CD_3SOCD_3, 80°C) δ 7.35 (s, 10H, $\mathbf{2C_6H_5}$); 5.83 (d, 1H, $\mathbf{H_1}$, $^3J_{1\text{-}2}$ = 3.9 Hz); 5.08 (s, 2H, $COOC\mathbf{H_2}$); 4.68 (d, 1H, $\mathbf{H_2}$, $^3J_{2\text{-}1}$ = 3.9 Hz); 4.57 (AB, 2H, $OC\mathbf{H_2}C_6H_5$; $^2J_{H\text{-}H}$ = 11.8 Hz); 4.22 (m, 1H, $\mathbf{H_4}$); 4.00 (h, 1H, $NC\mathbf{H}(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.8 Hz); 3.86 (d, 1H, $\mathbf{H_3}$, $^3J_{3\text{-}4}$ = 3.2 Hz); 3.06 (B of ABX, 1H, $\mathbf{H_{5a}}$, $^3J_{5a\text{-}4}$ = 3.6 Hz, $^2J_{5a\text{-}5b}$ = 15.1 Hz); 3.29 (A of ABX, 1H, $\mathbf{H_{5a}}$, $^3J_{5a\text{-}4}$ = 6.7 Hz, $^2J_{5a\text{-}5b}$ = 15.1 Hz); 1.34 (s, 3H, O-C($C\mathbf{H_3}$)$_2$-O); 1.26 (s, 3H, O-C($C\mathbf{H_3}$)$_2$-O); 1.15 (d, 3H, NCH($C\mathbf{H_3}$)$_2$, $^3J_{H\text{-}H}$ = 6.9 Hz); 1.12 (d, 3H, NCH($C\mathbf{H_3}$)$_2$, $^3J_{H\text{-}H}$ = 6.7 Hz);

^{13}C NMR (75.3 MHz, CD_3SOCD_3, 80°C) δ 168.33 (**C**=O); 137.57, 136.81, 128.06, 127.48, 127.43, 127.18 (aromatic **C**); 110.35 (O-**C**(CH_3)$_2$-O); 104.03 ($\mathbf{C_1}$); 81.60 ($\mathbf{C_2}$); 81.15 ($\mathbf{C_4}$); 79.33 ($\mathbf{C_3}$); 70.61 ($COOCH_2$); 65.83 (OCH_2); 48.30 ($\mathbf{C_5}$); 42.56 ($NCH(CH_3)_2$); 26.35, 25.92 (O-C($\mathbf{C}H_3$)$_2$-O); 20.25, 20.19 (NCH($\mathbf{C}H_3$)$_2$);

MS (FAB, NBA) m/z 456 (100.0%, $(M+H)^+$); 348 (61.2%, (M+1-BnOH));

$[\alpha]^D_{295}$ -28.3° (c 1.5, EtOAc).

Synthesis of 3-O-benzyl-5-isopropylamino-1,2-O-isopropylidenexylofuranose 182

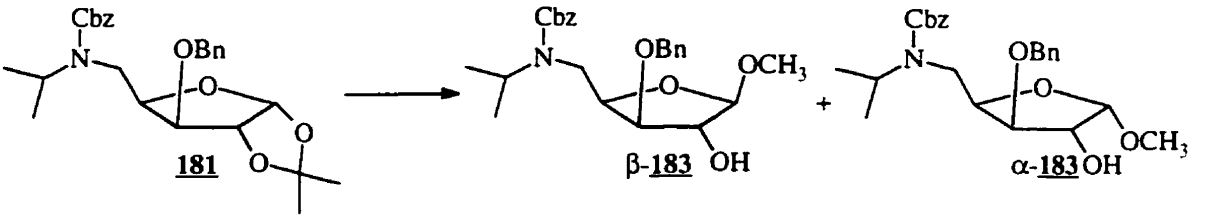


To a solution of 5-isopropylamino-1,2-O-isopropylidenexylofuranose **179** (3.41 mmol, 788 mg) stirred in dry THF at 0°C under Ar was added sodium hydride (4 mmol, 164 mg of a 60% suspension in mineral oil, not pre-washed with dry hexanes). After about 30 min, the hydrogen production stopped and benzyl bromide (3.41 mmol, 406 µl) was syringed in the solution over 5 min. Sodium iodide was then added (45 mg, 0.3 mmol), and the stirring was continued for 6h at 0°C, then at RT for overnight. After that period, a 10% solution of ammonium chloride was added dropwise into the solution (until the solution was clear) and the mixture was concentrated *in vacuo*. It was then poured into 100 ml of ethyl acetate, and washed twice with 20 ml of a 10% solution of sodium carbonate. The organic layer was dried over magnesium sulfate and evaporated to a yellow oily residue. The product was purified by flash chromatography (hexanes:ethyl acetate 90:10 to hexanes:ethyl acetate:triethylamine 50:45:5) to give 930 mg (85%) of pure **182** as a yellow oil. A small amount of the dibenzylated product was first collected (6%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.38 (m, 5H, $C_6\mathbf{H}_5$); 5.93 (d, 1H, $\mathbf{H}_{1'}$, $^3J_{1'\text{-}2'}$ = 4 Hz); 4.70 (B of AB, 1H, $OC\mathbf{H}_2$, $^2J_{H\text{-}H}$ = 12 Hz); 4.62 (d, 1H, $\mathbf{H}_{2'}$, $^3J_{2'\text{-}1'}$ = 4 Hz); 4.47 (A of AB, 1H, $OC\mathbf{H}_2$, $^2J_{H\text{-}H}$ = 12 Hz); 4.28 (ddd, 1H, $\mathbf{H}_{4'}$, $^3J_{4'\text{-}3'}$ = 3 Hz, $^3J_{4'\text{-}5'a}$ = 5 Hz, $^3J_{4'\text{-}5'b}$ = 8 Hz); 3.90 (d, 1H, $\mathbf{H}_{3'}$, $^3J_{3'\text{-}4'}$ = 3 Hz); 2.92 (B of ABX, 1H, $\mathbf{H}_{5'b}$, $^3J_{5'b\text{-}4'}$ = 8 Hz, $^2J_{5'b\text{-}5'a}$ = 12 Hz); 2.85 (A of ABX, 1H, $\mathbf{H}_{5'a}$, $^3J_{5'a\text{-}4'}$ = 5 Hz, $^2J_{5'b\text{-}5'a}$ = 12 Hz); 2.79 (h, 1H, $NC\mathbf{H}(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.5 Hz); 1.47 (s, 3H, O-C($C\mathbf{H}_3$)$_2$-O); 1.31 (s, 3H, O-C($C\mathbf{H}_3$)$_2$-O); 1.01 (t, 6H, NCH($C\mathbf{H}_3$)$_2$, $^3J_{H\text{-}H}$ = 6 Hz);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 137.41, 128.51, 127.98, 127.76 ($\mathbf{C}_6H_5$); 111.50 ($OC(CH_3)_2O$); 104.85 ($\mathbf{C}_{1'}$); 82.28 ($\mathbf{C}_{2'}$); 81.74 ($\mathbf{C}_{4'}$); 79.88 ($\mathbf{C}_{3'}$); 71.67 (OCH_2); 48.82 ($\mathbf{C}_{5'}$); 45.66 ($N\mathbf{C}(CH_3)_2$); 26.68, 26.24 (O-C($\mathbf{C}H_3$)$_2$-O); 23.02, 22.58 (NCH($\mathbf{C}H_3$)$_2$);

Synthesis of 1α- and 1β- 3-O-benzyl-5-deoxy-5-(N-carboxybenzyloxy-N-isopropylamino)-1-O-methyl xylofuranose 183



To a 1% solution of iodine (0.6 g) in dry methanol (60 ml) was added **181** (8.7 mmol, 3.95 g) and the mixture was refluxed under Ar and stirring until TLC indicated after 3-4h a complete reaction and formation of two products, a fast and a slow eluting one at R_f = 0.16 and R_f = 0.10 respectively (ethyl acetate:hexanes 30:70). The solution was then allowed to cool down to RT and a 1% solution of sodium thiosulfate was added dropwise until complete decolorization of the reaction mixture. The latter was concentrated *in vacuo* and taken up in 400 ml of ethyl acetate, washed twice with 50 ml of the same thiosulfate solution and with 50 ml brine. The organic layer was then dried over magnesium sulfate and evaporated *in vacuo* to a yellow oily residue. This oil was chromatographed using ethyl acetate:hexanes 15:85 to 20:80, to allow an almost complete separation of the two products. The fast eluting compound was obtained pure in 53.0% yield (1.98 g), the slow eluting one pure in 44% yield (1.64g).

fast-eluting isomer

^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 7.22-7.35 (m, 10H, **2C$_6$H$_5$**); 5.09 (B of AB, 1H, COOC**H**$_2$, $^2J_{H\text{-}H}$ = 12.5 Hz); 5.06 (A of AB, 1H, COOC**H**$_2$, $^2J_{H\text{-}H}$ = 12.5 Hz); 4.79 (d, 1H, **H**$_1$, $^3J_{1\text{-}2}$ = 4.5 Hz); 4.66 (B of AB, 1H, C$_3$OC**H**$_2$, $^2J_{H\text{-}H}$ = 11.5 Hz); 4.51 (A of AB, 1H, C$_3$OC**H**$_2$, $^2J_{H\text{-}H}$ = 11.5 Hz); 4.32 (b, exchangeable by D$_2$O, O**H**); 4.24 (ddd, 1H, **H**$_4$, $^3J_{4\text{-}5a}$ = 8.0 Hz, $^3J_{4\text{-}3}$ = 5.5 Hz, $^3J_{4\text{-}5b}$ = 3.0Hz); 4.09 (ddd, X of ABX, 1H, **H**$_2$, $^3J_{2\text{-}1}$ = 4.5 Hz, $^3J_{2\text{-}OH}$ = 4.5 Hz, $^3J_{2\text{-}3}$ = 6.0 Hz); 3.96 (h, 1H, NC**H**(CH$_3$)$_2$, $^3J_{H\text{-}H}$ = 7.0 Hz); 3.93 (dd, 1H, **H**$_3$, $^3J_{3\text{-}2}$ = 6.0 Hz, $^3J_{3\text{-}4}$ = 5.5 Hz); 3.59 (B of ABX, 1H, **H**$_{5b}$, $^2J_{5b\text{-}5a}$ = 15.0 Hz, $^3J_{5b\text{-}4}$ = 3.0 Hz); 3.31 (s, 3H, OC**H**$_3$); 3.24 (A of ABX, **H**$_{5a}$, $^2J_{5a\text{-}5b}$ = 15.0 Hz, $^3J_{5a\text{-}4}$ = 8.0 Hz); 1.19 (d, 3H, NCH(C**H**$_3$)$_2$, $^3J_{H\text{-}H}$ = 6.5 Hz); 1.17 (d, 3H, NCH(C**H**$_3$)$_2$, $^3J_{H\text{-}H}$ = 7.5 Hz);

^{13}C NMR (67.94 MHz, CD_3SOCD_3, 90°C) δ 155.62 (**C**=O); 138.88, 137.76, 128.83, 128.72, 128.16, 127.92 (aromatic **C**); 102.50 (**C_1**); 84.12 (**C_3**); 77.03 (**C_4**); 76.76 (**C_2**); 71.78 ($C_3O\mathbf{C}H_2C_6H_5$); 66.54 ($COO\mathbf{C}H_2C_6H_5$); 55.24 ($O\mathbf{C}H_3$); 49.44 ($N\mathbf{C}H(CH_3)_2$); 45.28 (**C_5**); 21.00, 20.82 ($CH(\mathbf{C}H_3)_2$;

MS (CI) m/z 430 (8.3%, $(M+1)^{+\cdot}$); 398 (10.1%, (M+1-CH_3OH)); 338 (36.6%, (M-Bn)); 206 (34.5%, (BnOCON(iPr)CH_2)); 162 (100.0%, (BnOCON=CH));

$[\alpha]^D_{295}$ +63.2° (c 1.2, methylene chloride)

slow-eluting isomer

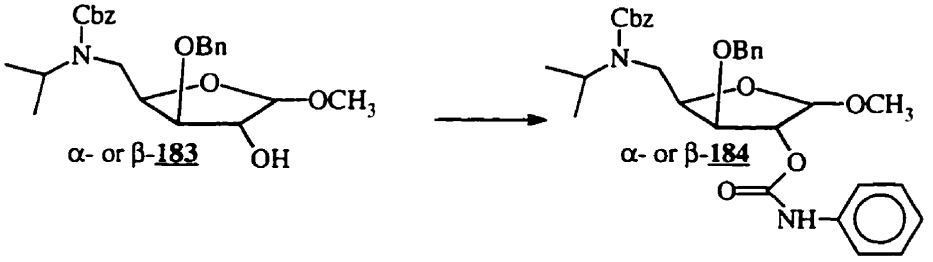
^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 7.26–7.38 (m, 10H, **2$C_6\mathbf{H}_5$**); 5.25 (d, 1H, O**H**, $^3J_{OH\text{-}H2}$ = 4.0 Hz); 5.07 (s, 2H, $COO\mathbf{C}\mathbf{H}_2$); 4.66 (d, 1H, **$H_1$**, $^3J_{1\text{-}2}$ = 2.0 Hz); 4.59 (B of AB, 1H, $C_3O\mathbf{C}\mathbf{H}_2$, $^2J_{H\text{-}H}$ = 12.0 Hz); 4.45 (A of AB, 1H, $C_3O\mathbf{C}\mathbf{H}_2$, $^2J_{H\text{-}H}$ = 12.0 Hz); 4.28 (ddd, X of ABX, 1H, **H_4**, $^3J_{4\text{-}3}$ = 5.5 Hz, $^3J_{4\text{-}5a}$ = 8.0 Hz, $^3J_{4\text{-}5b}$ = 2.5 Hz); 4.04 (ddd, 1H, **H_2**, $^3J_{2\text{-}1}$ = 2.0 Hz, $^3J_{2\text{-}OH}$ = 4.0 Hz, $^3J_{2\text{-}3}$ = 3.0 Hz); 3.94 (h, 1H, $N\mathbf{C}\mathbf{H}(CH_3)_2$, $^3J_{H\text{-}H}$ = 7.0 Hz); 3.79 (dd, 1H, **H_3**, $^3J_{3\text{-}4}$ = 5.5 Hz, $^3J_{3\text{-}2}$ = 3.0 Hz); 3.66 (B of ABX, 1H, **H_{5b}**, $^2J_{5b\text{-}5a}$ = 15.0 Hz, $^3J_{5b\text{-}4}$ = 2.5 Hz); 3.28 (s, 3H, $O\mathbf{C}\mathbf{H}_3$); 3.26 (A of ABX, 1H, **H_{5a}**, $^2J_{5a\text{-}5b}$ = 15.0 Hz, $^3J_{5a\text{-}4}$ = 8.0 Hz); 1.19 (d, 3H, $NCH(C\mathbf{H}_3)_2$, $^3J_{H\text{-}H}$ = 7.0 Hz); 1.16 (d, 3H, $NCH(C\mathbf{H}_3)_2$, $^3J_{H\text{-}H}$ = 7.0 Hz);

^{13}C NMR (67.94 MHz, CD_3SOCD_3, 90°C) δ 138.83, 137.72, 128.85, 128.70, 128.18, 127.96 (aromatic **C**); 110.40 (**C_1**); 84.19 (**C_3**); 80.42 (**C_4**); 78.43 (**C_2**); 71.59 ($C_3O\mathbf{C}H_2C_6H_5$); 66.56 ($COO\mathbf{C}H_2C_6H_5$); 55.51 ($O\mathbf{C}H_3$); 49.78 ($N\mathbf{C}H(CH_3)_2$); 46.13 (**C_5**); 21.07, 20.75 ($NCH(\mathbf{C}H_3)_2$);

MS (CI) m/z 430 (4.6%, $(M+H)^+$); 398 (20.3%, (M+1-CH_3OH)); 338 (44.3%, (M-Bn)); 206 (29.0%, (BnOCON(iPr)CH_2)); 162 (100.0%, (BnOCON=CH));

$[\alpha]^D_{295}$ -27.6° (c 2.5, methylene chloride).

Synthesis of 1α- and 1β- 3-O-benzyl-5-deoxy-5-(N-carboxybenzyloxy-N-isopropylamino)-1-O-methyl xylofuranose 184



To a solution of α- or β- 3-O-benzyl-2-carboxyphenylamino-5-deoxy-5-(N-carboxybenzyloxy-N-isopropylamino)-1-O-methyl xylofuranose **183** (425 mg, 1.0 mmol) in 10 ml dry pyridine, stirred at 0°C under Ar, was added triphosgene (104 mg, 0.35 mmol). The solution progressively turned pink, and the stirring was maintained for 1h at the same temperature. Then, aniline (103 µl, 1.1 mmol) was added into the mixture and the reaction was stirred at 0°C for an extra hour, then allowed to warm up to room temperature. It was stirred for 12h, then evaporated *in vacuo*. The residual sticky gum was dissolved in 40 ml ethyl acetate, washed twice with 5 ml of a 10% solution of ammonium chloride, then with 5 ml brine. The organic layer was dried over magnesium sulfate and evaporated to a yellow oily compound that was purified by flash chromatography to yield α-**184** (477 mg, 87.0%) or β-**184** (483 mg, 88.1%) as a clear oil.

α-**184**

^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 9.52 (b, 1H, N**H**); 7.47-7.00 (m, 15H, aromatic **H**); 5.11-5.04 (m, 4H, $\mathbf{H_1}$, $\mathbf{H_2}$, $NCO_2\mathbf{CH_2}C_6H_5$); 4.58 (AB, 2H, $C_3O\mathbf{CH_2}C_6H_5$, $^2J_{H\text{-}H}$ = 12.0 Hz); 4.27 (m, 1H, $\mathbf{H_4}$); 4.18 (m, 1H, $\mathbf{H_3}$); 3.99 (h, 1H, $N\mathbf{CH}(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.8 Hz); 3.63 (B of ABX, 1H, $\mathbf{H_{5b}}$, $^3J_{5b\text{-}4}$ = 2.7 Hz, $^2J_{5b\text{-}5a}$ = 15.1 Hz); 3.30 (A of ABX, 1H, $\mathbf{H_{5a}}$, $^3J_{5a\text{-}4}$ = 8.3 Hz, $^2J_{5a\text{-}5b}$ = 15.1 Hz); 3.27 (s, 3H, $O\mathbf{CH_3}$); 1.18, 1.16 (2d, 6H, $NCH(\mathbf{CH_3})_2$, $^3J_{H\text{-}H}$ = 6.8 Hz);

^{13}C NMR (75.3 MHz, CD_3SOCD_3, 90°C) δ 154.65, 152.18 (**C**=O); 138.45, 137.33, 136.70, 128.13, 127.82, 127.78, 127.17, 127.09, 127.03, 126.91, 122.18, 118.27 (aromatic **C**); 100.07 ($\mathbf{C_1}$); 80.49 ($\mathbf{C_3}$); 77.02 ($\mathbf{C_2}$); 75.96 ($\mathbf{C_4}$); 71.06 ($C_3O\mathbf{CH_2}C_6H_5$);

65.61 ($NCO_2CH_2C_6H_5$); 54.46 (OCH_3); 48.41 ($NCH(CH_3)_2$); 43.85 (**C_5**); 19.99, 19.83 ($NCH(CH_3)_2$);

MS (FAB, NBA) m/z 549 (0.2%, $(M+H)^+$); 517 (0.5%, (M+1-CH_3OH)); 206 (24.2%, (BnOCON(iPr)CH_2)); 162 (66.5%, (BnOCON=CH)); 119 (100.0%, C_6H_5NCO));

$[\alpha]^D_{295}$ +76.2° (c 0.85, ethyl acetate).

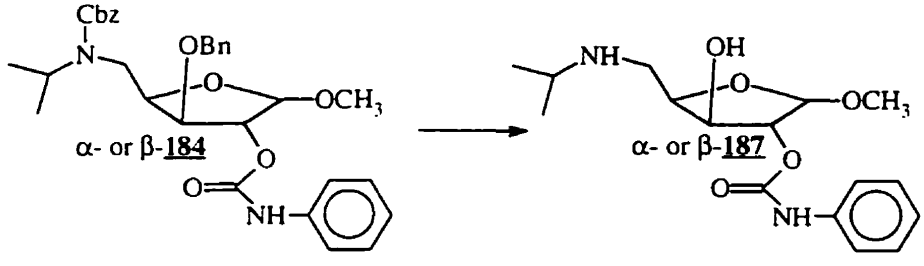
β-**184**

^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 9.55 (b, 1H, N**H**); 7.46-7.00 (m, 15H, aromatic **H**), 5.10 (m, 1H, $\mathbf{H_2}$); 5.07 (m, 2H, $NCO_2CH_2C_6H_5$); 4.91 (m, 1H, $\mathbf{H_1}$); 4.61 (AB, 2H, $C_3OCH_2C_6H_5$, $^2J_{H\text{-}H}$ = 12.0 Hz); 4.33 (m, 1H, $\mathbf{H_4}$); 4.00 (m, 1H, $\mathbf{H_3}$); 3.97 (h, 1H, $NCH(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.6 Hz); 3.67 (B of ABX, 1H, $\mathbf{H_{5b}}$, $^3J_{5b\text{-}4}$ = 2.93 Hz, $^2J_{5b\text{-}5a}$ = 15.1 Hz); 3.33 (s, 3H, OCH_3); 3.30 (A of ABX, 1H, $\mathbf{H_{5a}}$, $^3J_{5a\text{-}4}$ = 7.6 Hz, $^2J_{5a\text{-}5b}$ = 15.1 Hz); 1.20, 1.16 (2d, 6H, $NCH(CH_3)_2$, $^3J_{H\text{-}H}$ = 6.6 Hz);

^{13}C NMR (75.3 MHz, CD_3SOCD_3) δ 154.70, 152.04 (**C**=O); 138.47, 137.61, 136.80, 128.51, 128.35, 128.12, 128.04, 127.49, 127.37, 127.24, 122.60, 118.36 (aromatic **C**); 106.81 (**C_1**); 82.15 (**C_3**); 80.60 (**C_2**); 79.20 (**C_4**); 70.87 (**$C_3OCH_2C_6H_5$**); 65.85 ($NCO_2CH_2C_6H_5$); 54.82 (OCH_3); 48.76 ($NCH(CH_3)_2$); 44.92 (**C_5**); 20.32, 20.04 ($NCH(CH_3)_2$);

$[\alpha]^D_{295}$ -55.0° (c 0.80, ethyl acetate).

Synthesis of 2-O-carboxyphenylamino-5-deoxy-5-N-isopropylamino-1-O-methyl-xylofuranose 187



A solution of α- or β-**184** (548 mg, 1.0 mmol) and Pd/C 10% (660 mg) in a mixture of ethanol (25 ml), water (3 ml) and acetic acid (3 ml) was shaken, under a pressure vessel, under a pressure of 55 psi of gaseous hydrogen for 36 h. After that time,

the mixture was carefully filtered over a short very compact pad of celite and evaporated to a yellow oil. Flash chromatography gave the corresponding α-**187** as a white solid (m. p. 131-133°C) or β-**187** as a colorless oil in 77% yield (250 mg).

α-**187**

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.06 (m, 5H, $C_6\mathbf{H}_5$); 5.04 (m, 1H, $\mathbf{H}_1$); 4.97 (m, 1H, $\mathbf{H}_2$); 4.40 (m 1H, $\mathbf{H}_4$); 4.31 (d, $\mathbf{H}_3$, $^3J_{3-4}$ = 5.1 Hz); 3.47 (s, 3H, $OC\mathbf{H}_3$); 2.21 (B of ABX, $\mathbf{H}_{5b}$, 1H, $^2J_{5b-5a}$ = 12.5 Hz, $^3J_{5b-4}$ = 4.3 Hz); 2.97 (A of ABX, $\mathbf{H}_{5a}$, 1H, $^2J_{5a-5b}$ = 12.5 Hz, $^3J_{5a-4}$ = 2.5 Hz); 2.79 (h, 1H, $NC\mathbf{H}(CH_3)_2$, $^3J_{H-H}$ = 6.4 Hz); 1.10, 1.09 (2d, $NCH(C\mathbf{H}_3)_2$, 6H, $^3J_{H-H}$ = 6.4 Hz);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 152.14 (**C**=O); 129.11, 123.82, 123.79, 118.56 (aromatic **C**); 107.70 ($\mathbf{C}_1$); 83.49 ($\mathbf{C}_3$); 80.29 ($\mathbf{C}_2$); 76.58 ($\mathbf{C}_4$); 55.86 (OCH_3); 48.67 ($NCH(CH_3)_2$); 46.57 ($\mathbf{C}_5$); 22.73, 22.33 ($NCH(\mathbf{C}H_3)_2$);

MS (CI) m/z 325 (16.2 %, $(M+H)^+$); 206 (59.1%, $(M+1-C_6H_5NCO)$); 119 (100.0%, (C_6H_5NCO));

$[\alpha]^D_{295}$ +184.0° (c 0.80, MeOH).

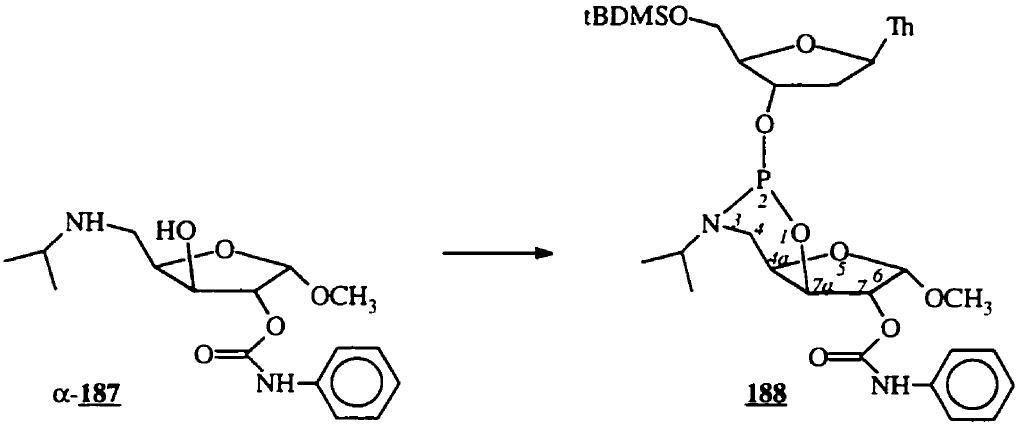
β-**187**

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.77 (b, 1H, OCON**H**); 7.57-7.08 (m, 5H, $C_6\mathbf{H}_5$); 5.20 (d, 1H, $\mathbf{H}_1$, $^3J_{1-2}$ = 4.0 Hz); 4.827 (dd, 1H, $\mathbf{H}_2$, $^3J_{2-1}$ = 4.0 Hz, $^3J_{2-3}$ = 3.7 Hz); 4.08 (dd, 1H, $\mathbf{H}_3$, $^3J_{3-2}$ = 3.7 Hz, $^3J_{3-4}$ = 6.1 Hz); 3.93 (m, 1H, $\mathbf{H}_4$); 3.42 (s, 3H, $OC\mathbf{H}_3$); 3.11 (B of ABX, 1H, $\mathbf{H}_{5b}$, $^2J_{5b-5a}$ = 12.7 Hz, $^2J_{5b-4}$ = 3.5 Hz); 2.80 (A of ABX, 1H, $\mathbf{H}_{5a}$, $^2J_{5a-5b}$ = 12.7 Hz, $^3J_{5a-4}$ = 1.7 Hz); 2.74 (h, 1H, $NC\mathbf{H}(CH_3)_2$, $^3J_{H-H}$ = 6.8 Hz); 1.07, 1.04 (2d, 6H, $NCH(C\mathbf{H}_3)_2$, $^3J_{H-H}$ = 6.8 Hz);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 155.16 (**C**=O); 137.60, 128.79, 123.83, 119.70 (aromatic **C**); 100.84 ($\mathbf{C}_1$); 83.36 ($\mathbf{C}_3$); 75.81 ($\mathbf{C}_2$), 74.79 ($\mathbf{C}_4$), 55.80 (OCH_3), 48.48 ($NCH(CH_3)_2$), 45.84 ($\mathbf{C}_5$), 22.61, 22.13 ($NCH(\mathbf{C}H_3)_2$);

$[\alpha]^D_{295}$ -89.7° (c 0.88, MeOH).

Synthesis of (4a*R*, 6*S*,7*S*, 7a*S*)-2-(5'-*ter*butyldimethylsilyl)thymid-3'-yl-3-isopropyl-6-methoxy-7-phenylaminocarboxyl-4a,6,7,7a-tetrahydrofuro[2,3-e]-oxazaphosphorinane 188



To a solution of freshly distilled phosphorus trichloride (87 µl, 1.0 mmol) in 2 ml dry chloroform stirred at 0 under Ar, was added a mixture of α-**187** (325 mg, 1.0 mmol) and triethylamine (349 µl, 2.5 mmol) in 3 ml dry chloroform, over a period of time of 1h. After the addition, ^{31}P NMR indicated the presence of many signals in the area 140-170 ppm. The mixture was refluxed for 4h, after which time ^{31}P NMR indicated the presence of a few compounds, characterized by a signal at 149.5 ppm. refluxing for 48h did not improve this ratio in a previous experiment, and simply led to a large amount of decomposition. Therefore after 4h, the mixture was cooled down to 0°C and a mixture of 5'-O-tBDMS-thymidine (356 mg, 1.0 mmol) and dry triethylamine (168 µl, 1.2 mmol) in 3 ml dry chloroform was injected into the reaction mixture, over 5 min. The mixture was refluxed again, and after 24 to 48h ^{31}P NMR indicated the presence of a single compound having a resonance frequency at 136.1 ppm , along with about 20% decomposition products appearing around 10 ppm. To the mixture cooled down to RT was added 20 ml of ethyl acetate (pre-washed twice with a saturated solution of sodium bicarbonate and dried over magnesium sulfate) to precipitate triethylammonium chloride. The salt was filtered out and the solution concentrated *in vacuo*. The crude mixture was purified by flash chromatography, using hexanes:ethyl acetate:triethylamine (60:25:15 to 45:40:15) as an eluent. Pure **188** could thus be obtained in 55 to 60% yield (390 to 425 mg) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (s, 1H, C=C**H**); 7.38-6.91 (m, 5H, aromatic **H**); 6.34 (dd, 1H, $\mathbf{H_{1'}}$, $^3J_{1'\text{-}2'b}$ = 5.6 Hz, $^3J_{1'\text{-}2'a}$ = 8.3 Hz); 5.21 (d, 1H, $\mathbf{H_1}$, $^3J_{1\text{-}2}$ = 4.4 Hz); 5.14 (m, 1H, $\mathbf{H_2}$); 4.64 (m, 1H, $\mathbf{H_{3'}}$); 4.54 (m, 1H, $\mathbf{H_3}$); 4.27 (m, 1H, $\mathbf{H_4}$); 4.07 (m, 1H, $\mathbf{H_{4'}}$); 3.86 (AB of ABX, 2H, $\mathbf{H_{5'b}}$, $\mathbf{H_{5'a}}$, $^2J_{5'b\text{-}5'a}$ = 11.5 Hz, $^3J_{5'b\text{-}4'}$ = 2.0 Hz, $^3J_{5'a\text{-}4'}$ = 2.2 Hz); 3.45 (s, 3H, $OC\mathbf{H_3}$); 3.42-3.35 (m, 2H, $\mathbf{H_{5b}}$, $NC\mathbf{H}(CH_3)_2$); 2.93 (dt, 1H, $\mathbf{H_{5a}}$, $^2J_{5a\text{-}5b}$ = 13.4 Hz, $^3J_{5a\text{-}4}$ = 5.1 Hz, $^3J_{5a\text{-}P}$ = 5.1 Hz); 2.38 (ddd, 1H, $\mathbf{H_{2'b}}$, $^2J_{2'b\text{-}2'a}$ = 13.4 Hz, $^3J_{2'b\text{-}1'}$ = 5.6 Hz, $^3J_{2'b\text{-}3'}$ = 2.0 Hz); 2.10 (ddd, 1H, $\mathbf{H_{2'a}}$, $^2J_{2'a\text{-}2'b}$ = 13.4 Hz, $^3J_{2'a\text{-}1'}$ = 8.3 Hz, $^3J_{2'a\text{-}3'}$ = 6.1 Hz); 1.91 (s, 3H, CH=CC$\mathbf{H_3}$); 1.15 (2d, 6H, NCH(C$\mathbf{H_3}$)$_2$, $^3J_{H\text{-}H}$ = 6.3 Hz); 0.91 (s, 9H, SiC(C$\mathbf{H_3}$)$_3$); 0.11 (s, 6H, Si(C$\mathbf{H_3}$)$_2$);

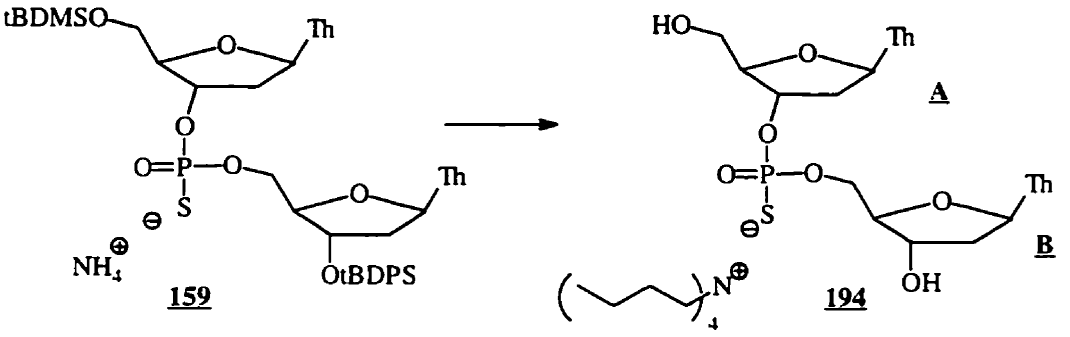
^{13}C NMR (67.94 MHz, $CDCl_3$) δ 163.20 (NH**C**=OC(CH_3)); 152.29 (C_2**O**C=ONHC_6H_5); 150.12 (N**C**=ONH); 135.16 (N**C**H=C(CH_3); 137.72, 129.03, 123.75, 119.00 (NHC=O**C**$_6$H$_5$); 110.83 (NCH=**C**(CH_3)); 102.03 ($\mathbf{C_1}$); 86.59 ($\mathbf{C_{4'}}$, d, $^3J_{C4'\text{-}P}$ = 3.6 Hz); 85.01 ($\mathbf{C_{1'}}$); 78.89 ($\mathbf{C_2}$, d, $^3J_{C2\text{-}P}$ = 2.1 Hz); 73.40 ($\mathbf{C_{3'}}$, d, $^2J_{C3'\text{-}P}$ = 19.7 Hz); 73.09 ($\mathbf{C_3}$, d, $^2J_{C3\text{-}P}$ = 4.7 Hz); 72.98 ($\mathbf{C_4}$, d, $^3J_{C4\text{-}P}$ = 4.1 Hz); 63.12 ($\mathbf{C_{5'}}$); 56.16 ($O\mathbf{C}H_3$); 49.79 (NCH(CH_3)$_2$, d, $^2J_{C\text{-}P}$ = 34 Hz); 46.42 ($\mathbf{C_5}$); 40.38 ($\mathbf{C_{2'}}$, d, $^3J_{C2'\text{-}P}$ = 5.2 Hz); 25.95 (SiC(**C**H$_3$)$_3$); 22.11 (NCH(**C**H$_3$)$_2$, d, $^3J_{C\text{-}P}$ = 9.3 Hz); 21.72 (NCH(**C**H$_3$)$_2$, d, $^3J_{C\text{-}P}$ = 5.1 Hz); 18.34 (Si**C**(CH$_3$)$_3$); 12.25 (CH=C(**C**H$_3$)); -5.39, -5.43 (Si(**C**H$_3$)$_2$)

^{31}P NMR (109.38 MHz, $CDCl_3$) δ 136.1;

MS (FAB, NBA + NaCl) m/z 731 (6.3%, $(M+Na)^+$); 353 (44.5%, (M+1-(5'-O-tBDMS-thymidine))); 339 (100.0%, (5'-O-tBDMS-thymidine+1-H_2O));

HRMS (FAB) $(M+Na)^+$ $C_{32}H_{49}N_4O_{10}SiPNa$ (calc. 731.285331, found 731.285460).

Synthesis of tetrabutylammonium thymid-3'-yl thymid-5'-yl phosphorothioate 194



To a solution of ammonium (5'-O-terbutyldimethylsilyl)-thymid-3'-yl (3'-O-terbutyldiphenylsilyl)thymid-5'-yl phosphorothioate **159** (50 mg, 0.054 mmol) in 1 ml dry DMF was added a 1M solution of TBAF in THF (135 µl, 0.135 mmol). After 3h, TLC indicated the reaction had gone to completion. Therefore the solvents were evaporated *in vacuo*, and the desired dimer was purified by silica gel chromatography, using a short column and polar eluent: acetone to acetone:water 98:2. The product was obtained in 89.9 % yield (39 mg). Each nucleoside unit is referred to as A and B as indicated in the scheme.

^{1}H NMR (500 MHz, CD_3OD) δ 7.90 (d, 1H, C**H**=C(CH_3); $^4J_{H\text{-}H}$ = 1.0 Hz); 7.85 (d, 1H, C**H**=C(CH_3), $^4J_{H\text{-}H}$ = 1.0 Hz); 6.35 (dd, 1H, $\mathbf{H_{1'B}}$, $^3J_{1'\text{-}2'}$ = 8.1 Hz, $^3J_{1'\text{-}2'}$ = 6.1 Hz); 6.28 (dd, 1H, $\mathbf{H_{1'A}}$, $^3J_{1'\text{-}2'}$ = 8.3 Hz, $^3J_{1'\text{-}2'}$ = 5.6 Hz); 5.06 (m, 1H, $\mathbf{H_{3'A}}$); 4.51 (m, 1H, $\mathbf{H_{3'B}}$); 4.22 (m, 1H, $\mathbf{H_{4'A}}$); 4.13 (AB of ABX dedoubled, 1H, $\mathbf{2xH_{5'B}}$, $^2J_{5'b\text{-}5'a}$ = 11.3 Hz, $^3J_{5'b\text{-}4'}$ = 2.9 Hz, $^3J_{5'a\text{-}4'}$ = 2.7 Hz, $^3J_{5'b\text{-}P}$ = 6.6 Hz, $^3J_{5'a\text{-}P}$ = 5.6 Hz); 4.04 (m, 1H, $\mathbf{H_{4'B}}$); 3.82 (AB of ABX, 1H, $\mathbf{2xH_{5'a}}$, $^2J_{5'a\text{-}5'b}$ = 12.0 Hz, $^3J_{5'b\text{-}4'}$ = 3.2 Hz, $^3J_{5'a\text{-}4'}$ = 2.9 Hz); 3.23 (m, 8H, N($C\mathbf{H_2}$Pr)$_4$); 2.46 (B of ABX dedoubled, 1H, $\mathbf{H_{2'A}}$, $^2J_{2'b\text{-}2'a}$ = 13.7 Hz, $^3J_{2'b\text{-}1'}$ = 5.6 Hz, $^3J_{2'b\text{-}3'}$ = 2.2 Hz); 2.31-2.16 (m, 3H, $\mathbf{2xH_{5'B}}$, $\mathbf{H_{5'A}}$); 1.97 (d, 3H, CH=C($C\mathbf{H_3}$), $^4J_{H\text{-}H}$ = 1.0 Hz); 1.86 (d, 3H, CH=C($C\mathbf{H_3}$), $^4J_{H\text{-}H}$ = 1.0 Hz); 1.65 (tq, 8H, N($CH_2C\mathbf{H_2}$Et)$_4$, $^3J_{H\text{-}H}$ = 7.6 Hz, $^3J_{H\text{-}H}$ = 8.3 Hz); 1.41 (tq, 8H, N($CH_2CH_2C\mathbf{H_2}CH_3$)$_4$, $^3J_{H\text{-}H}$ = 7.3 Hz, $^3J_{H\text{-}H}$ = 7.6 Hz); 1.01 (t, 12H, N($CH_2CH_2CH_2C\mathbf{H_3}$)$_4$, $^3J_{H\text{-}H}$ = 7.3 Hz);

^{13}C NMR (125.7 MHz, CD_3OD) δ 166.55, 166.42 (2xNHC=OC(CH_3)); 152.47, 152.29 (2xNC=ONH); 138.20, 138.12 (2xCH=C(CH_3)); 112.09, 111.55 (2xNCH=C(CH_3)); 87.93 (d, $\mathbf{C_{4'A}}$, $^3J_{4'A}$= = 5.5 Hz); 87.49 (d, $\mathbf{C_{4'B}}$, $^3J_{4'B\text{-}P}$ = 8.2 Hz); 86.29 ($\mathbf{C_{1'A}}$); 86.16 ($\mathbf{C_{1'B}}$); 77.73 (d, $\mathbf{C_{3'A}}$, $^2J_{3'A\text{-}P}$ = 5.5 Hz); 72.98 ($\mathbf{C_{3'B}}$); 66.40 (d, $\mathbf{C_{5'B}}$, $^2J_{5'B\text{-}P}$ = 6.4 Hz);

62.94 ($C_{5'A}$); 59.49 (t, $N(CH_2Pr)_4$); 40.97 ($C_{2'B}$); 39.94 (d, $C_{2'A}$, $^3J_{2'A-P}$ = 4.6 Hz); 24.78 ($N(CH_2CH_2Et)_4$); 20.72 ($N(CH_2CH_2CH_2CH_3)_4$); 13.94 ($N(CH_2CH_2CH_2CH_3)_4$); 12.70, 12.48 (2xCH=C(CH_3));

MS (FAB +, NBA) m/z 242 (100.0%, $(NBu_4)^+$);

MS (FAB -, NBA) m/z 561 $(dTP(S)(O)dT)^-$);

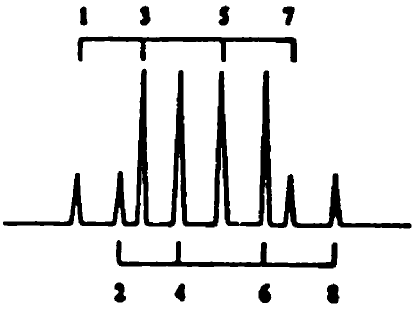
HRMS (FAB +) $(M+Na)^+$ $C_{20}H_{27}N_4O_{11}PSNa$ (calc. 585.103238, found 585.103400).

7. Appendix

Appendix 1: Analysis of ABX systems in ^{1}H NMR spectra[1]

Chemical shifts and coupling constants of the AB part of ABX spectra were calculated according to the method presented below.

AB #1



AB #2

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

AB #1

$$\delta_1 = (1 + 3 + 5 + 7) / 4$$
$$(\Delta\delta_1) / 2 = [(1 - 7)(3 - 5)]^{1/2} / 2$$
$$\Delta 1^+ = \delta_1 + (\Delta\delta_1) / 2$$
$$\Delta 1^- = \delta_1 - (\Delta\delta_1) / 2$$

$$\delta_A = (\Delta 1^+ + \Delta 2^+) / 2$$
$$J_{AX} = \Delta 1^+ - \Delta 2^+$$

or

$$\delta_A = (\Delta 1^+ + \Delta 2^-) / 2$$
$$J_{AX} = \Delta 1^+ - \Delta 2^-$$

AB #2

$$\delta_2 = (2 + 4 + 6 + 8) / 4$$
$$(\Delta\delta_2) / 2 = [(2 - 8)(4 - 6)]^{1/2} / 2$$
$$\Delta 2^+ = \delta_2 + (\Delta\delta_2) / 2$$
$$\Delta 2^- = \delta_2 - (\Delta\delta_2) / 2$$

$$\delta_B = (\Delta 1^- + \Delta 2^-) / 2$$
$$J_{BX} = \Delta 1^- - \Delta 2^-$$

or

$$\delta_B = (\Delta 1^- + \Delta 2^+) / 2$$
$$J_{BX} = \Delta 1^- - \Delta 2^+$$

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

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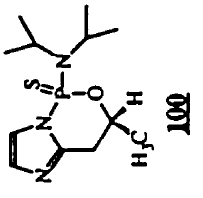
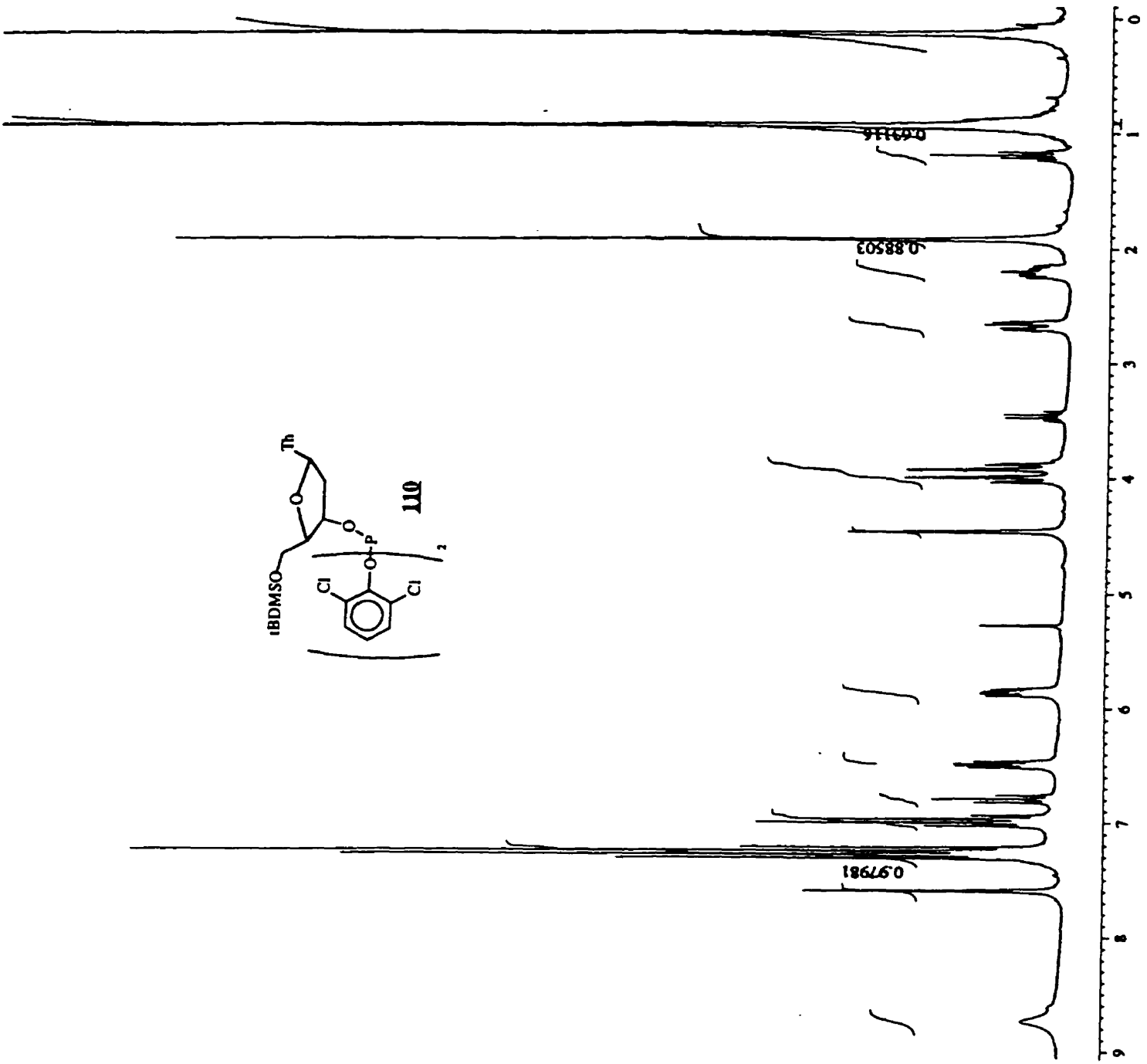
[1] "*High Resolution NMR- Theory and Applications*", Becker, E. D. ed., 1980, Academic Press, Inc. chap.7

Appendix 2: ^{1}H NMR spectrum (270 MHz, $CDCl_3$) of imidazo-oxazaphosphorine **100**



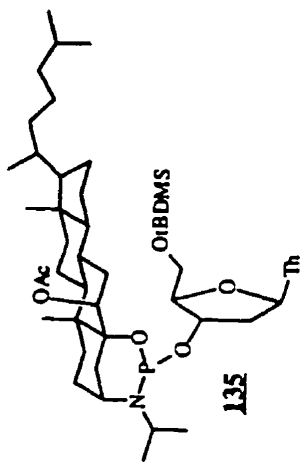
100

Appendix 3: ^{1}H NMR spectrum (270 MHz, $CDCl_3$) of activated phosphite triester **110**



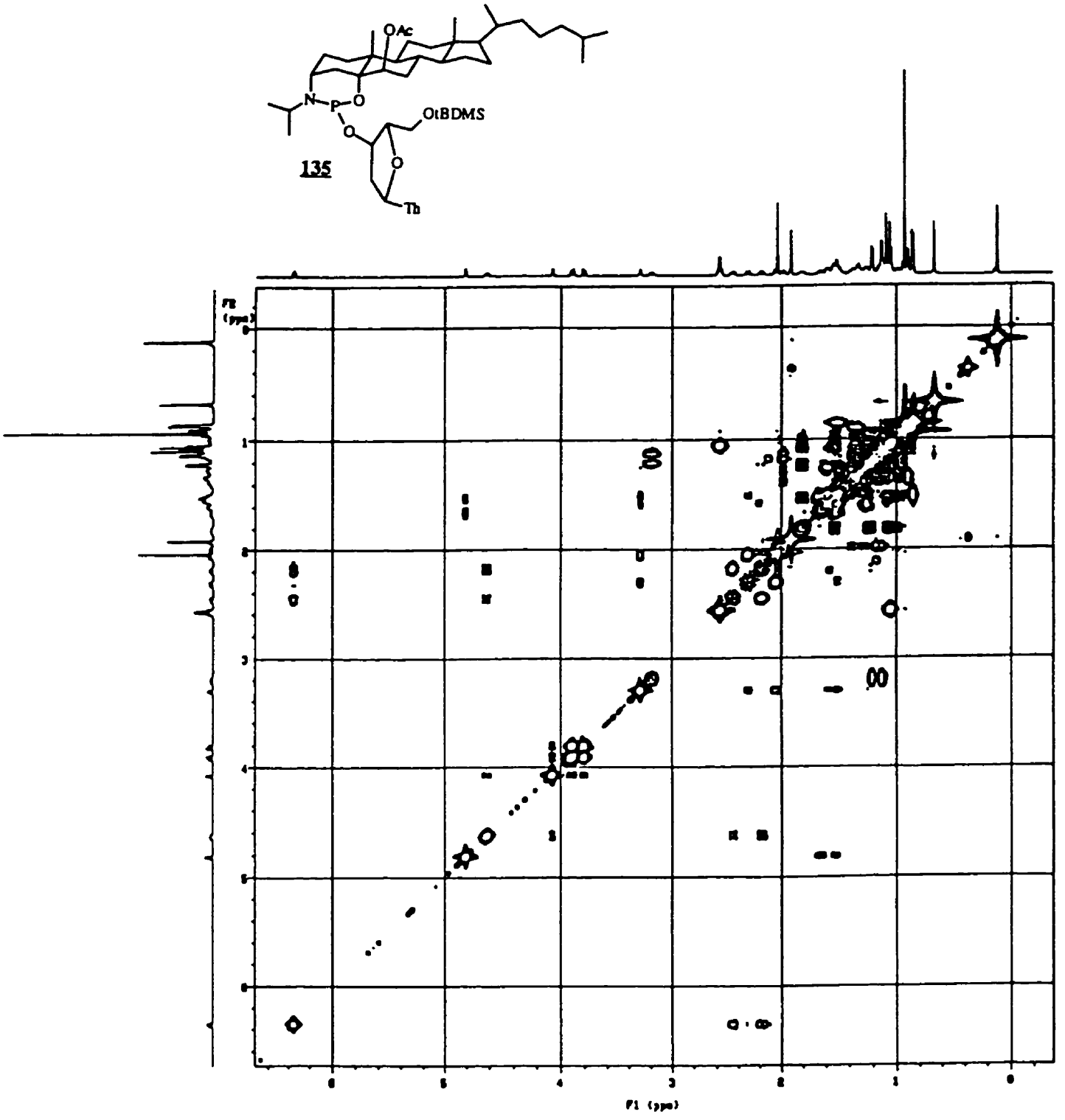
Appendix 4: 2D-COSY map (270 MHz, $CDCl_3$) of activated phosphite triester **110**

Appendix 5: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of oxazaphosphorinane <u>135</u>

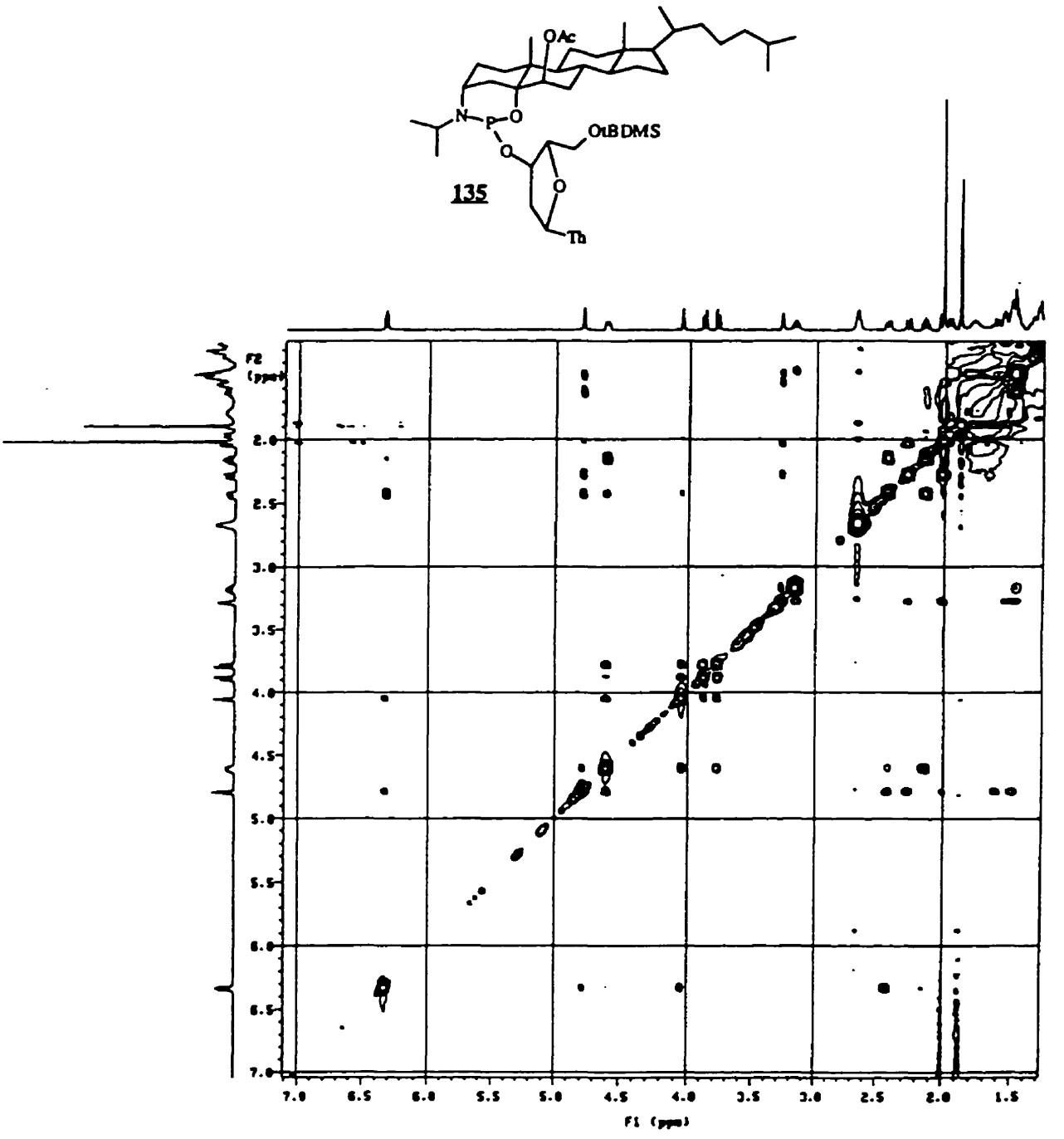


<u>135</u>

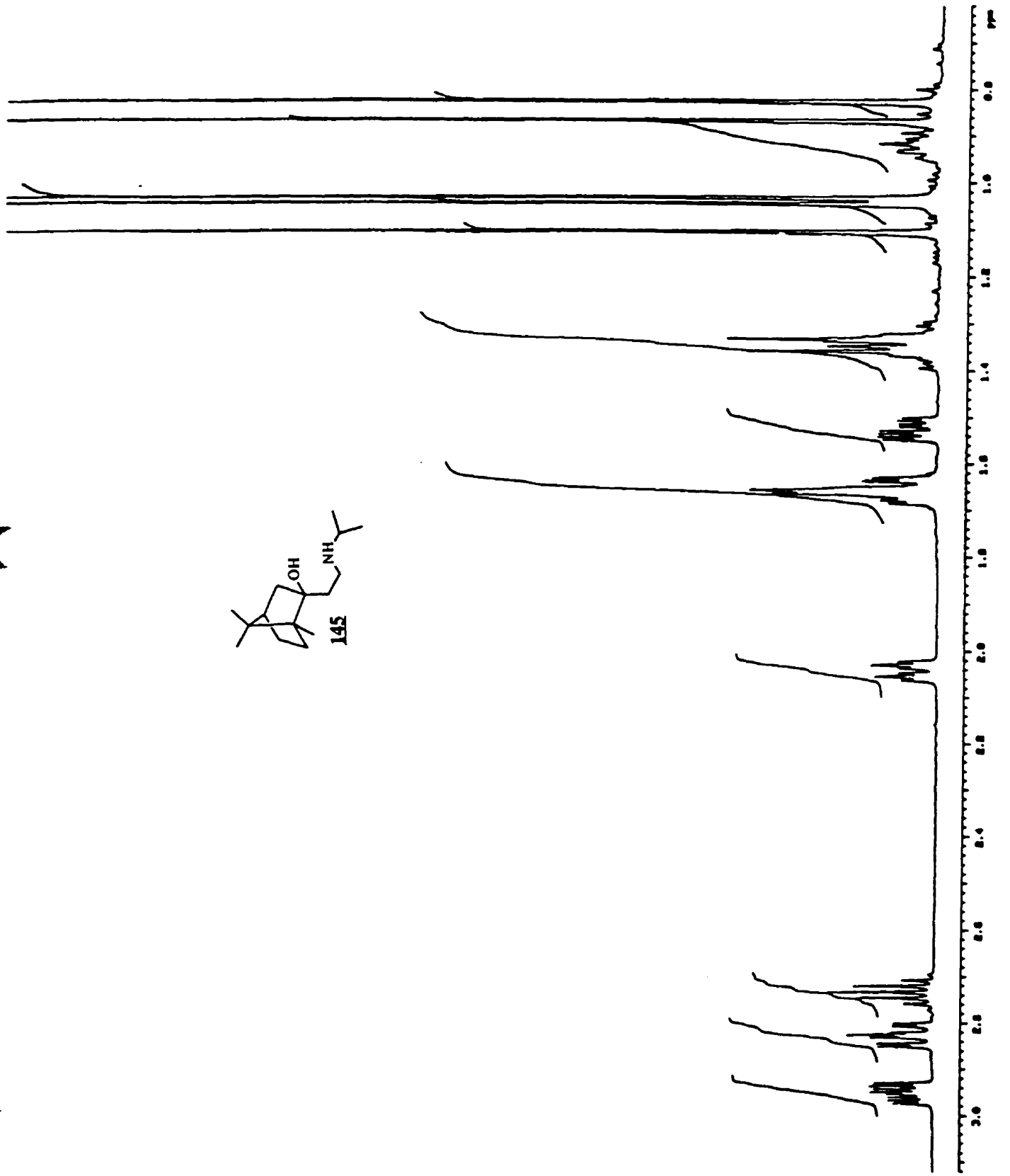
Appendix 6: 2D-COSY map (500 MHz, $CDCl_3$) of oxazaphosphorinane <u>135</u>



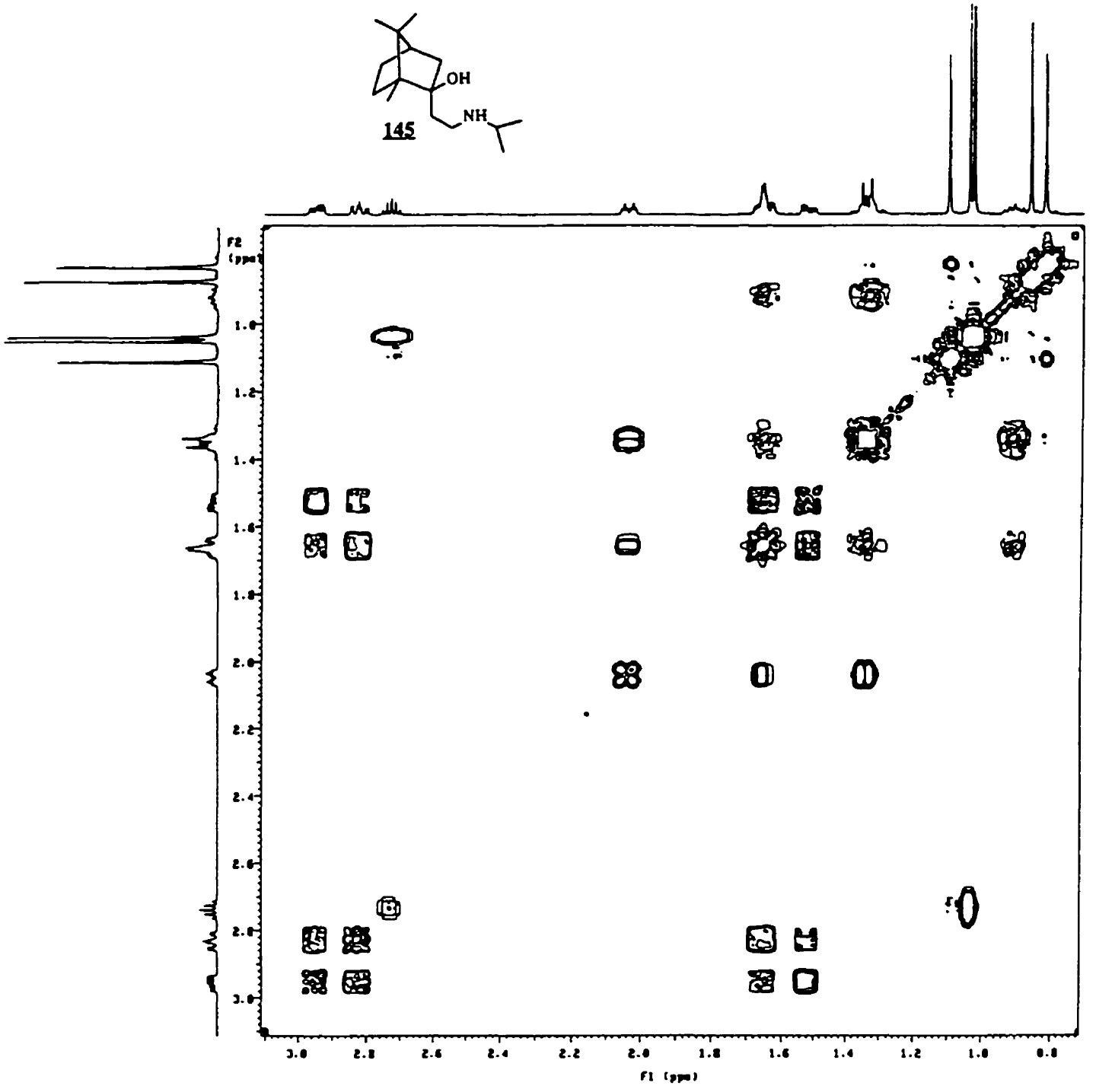
Appendix 7: 2D-NOE map (500 MHz, $CDCl_3$, mix.= 1200 msec.) of oxazaphosphorinane **135**



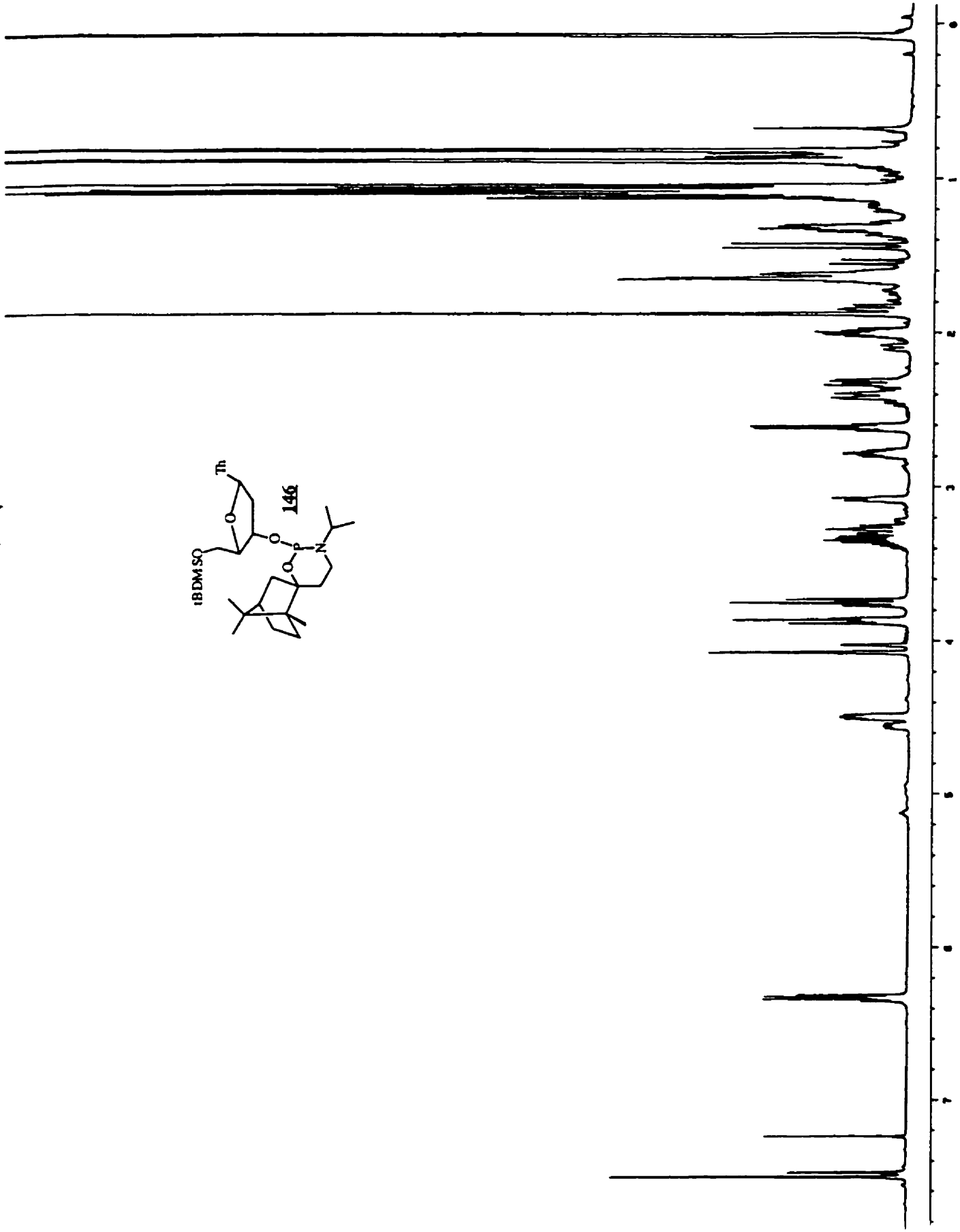
Appendix 8: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of γ-aminoalcohol **145**



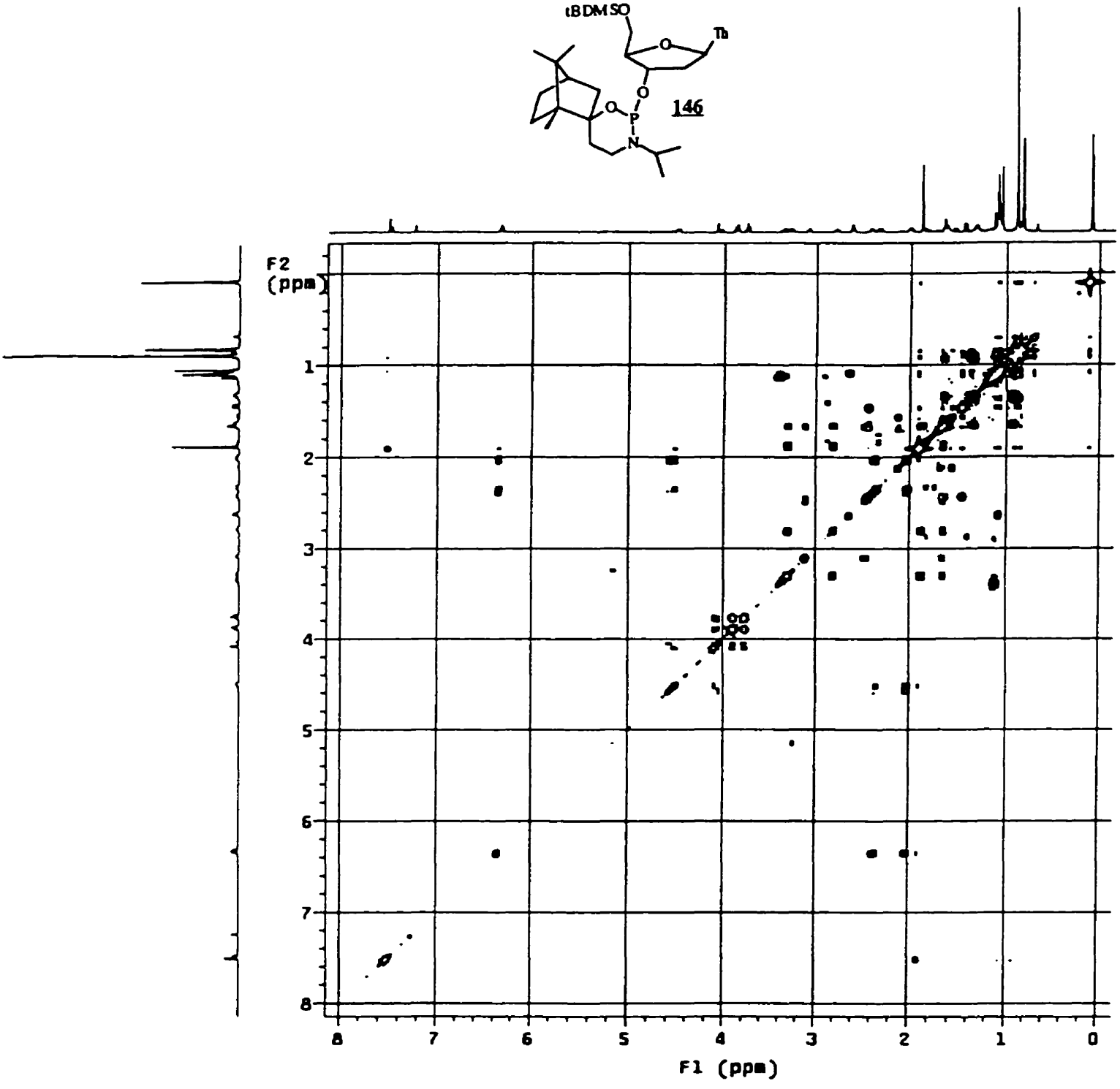
Appendix 9: 2D-COSY map (500 MHz, $CDCl_3$) of γ-aminoalcohol **145**



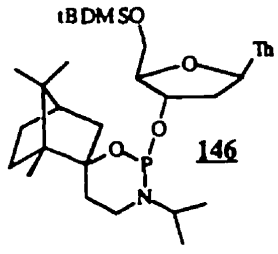
Appendix 10: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of oxazaphosphorinane **146** (2:1 mixture of diastereomers)



Appendix 11: 2D-COSY map (500 MHz, $CDCl_3$) of oxazaphosphorinane <u>146</u> (2:1 mixture of diastereomers

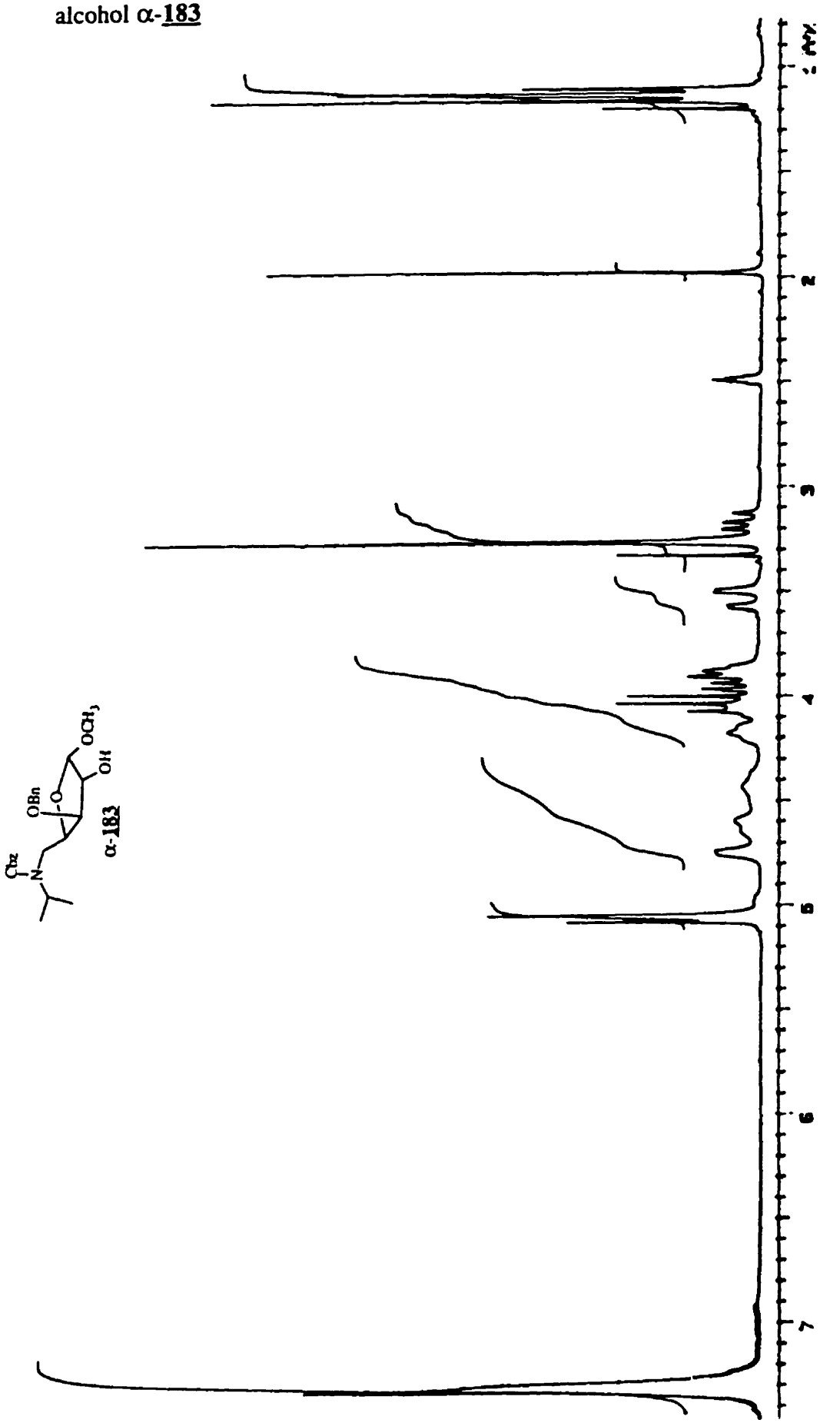


Appendix 12: 2D-HMQC map (500 MHz, $CDCl_3$) of oxazaphosphorinane **146** (2:1 mixture of diastereomers)

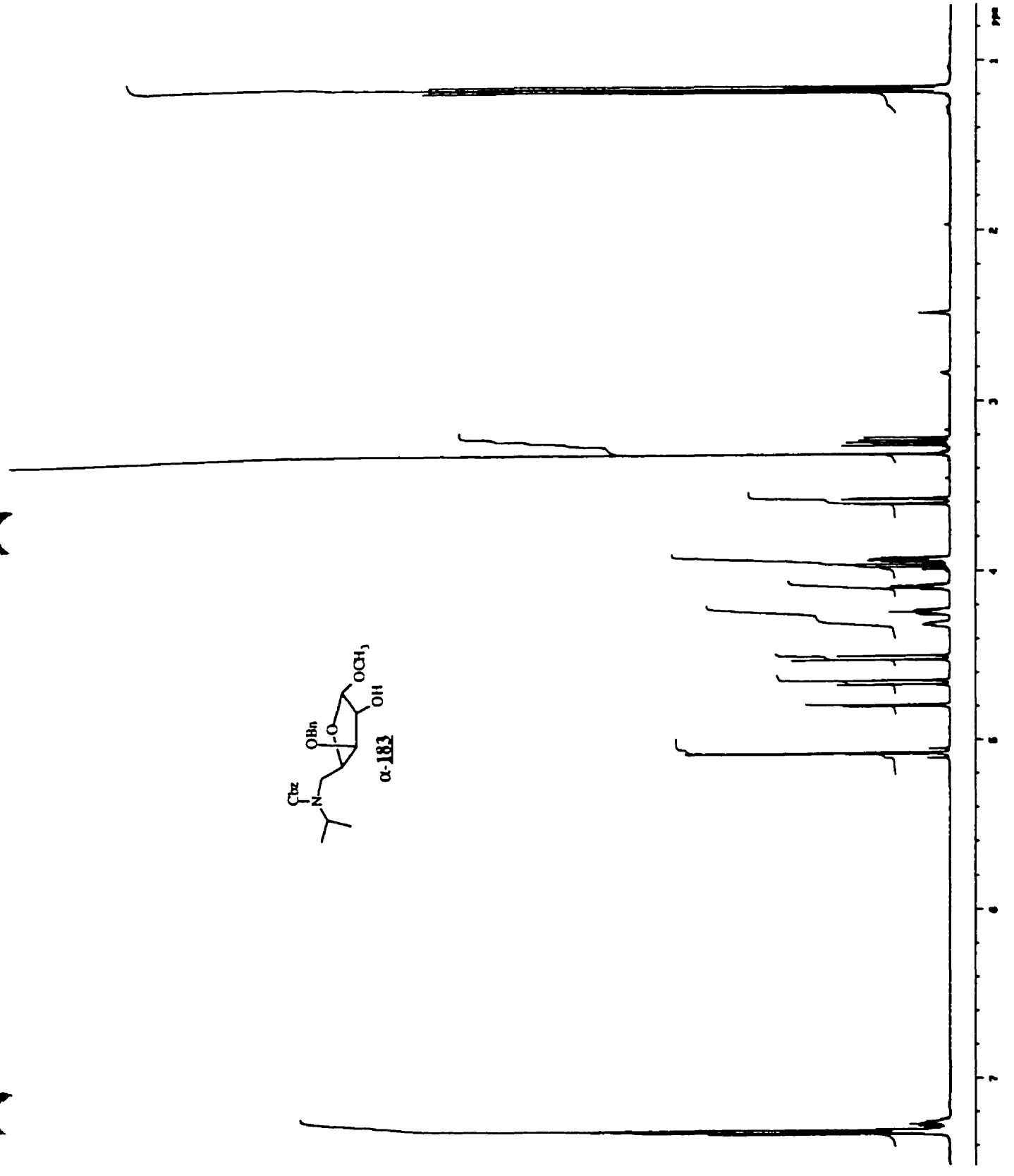


146

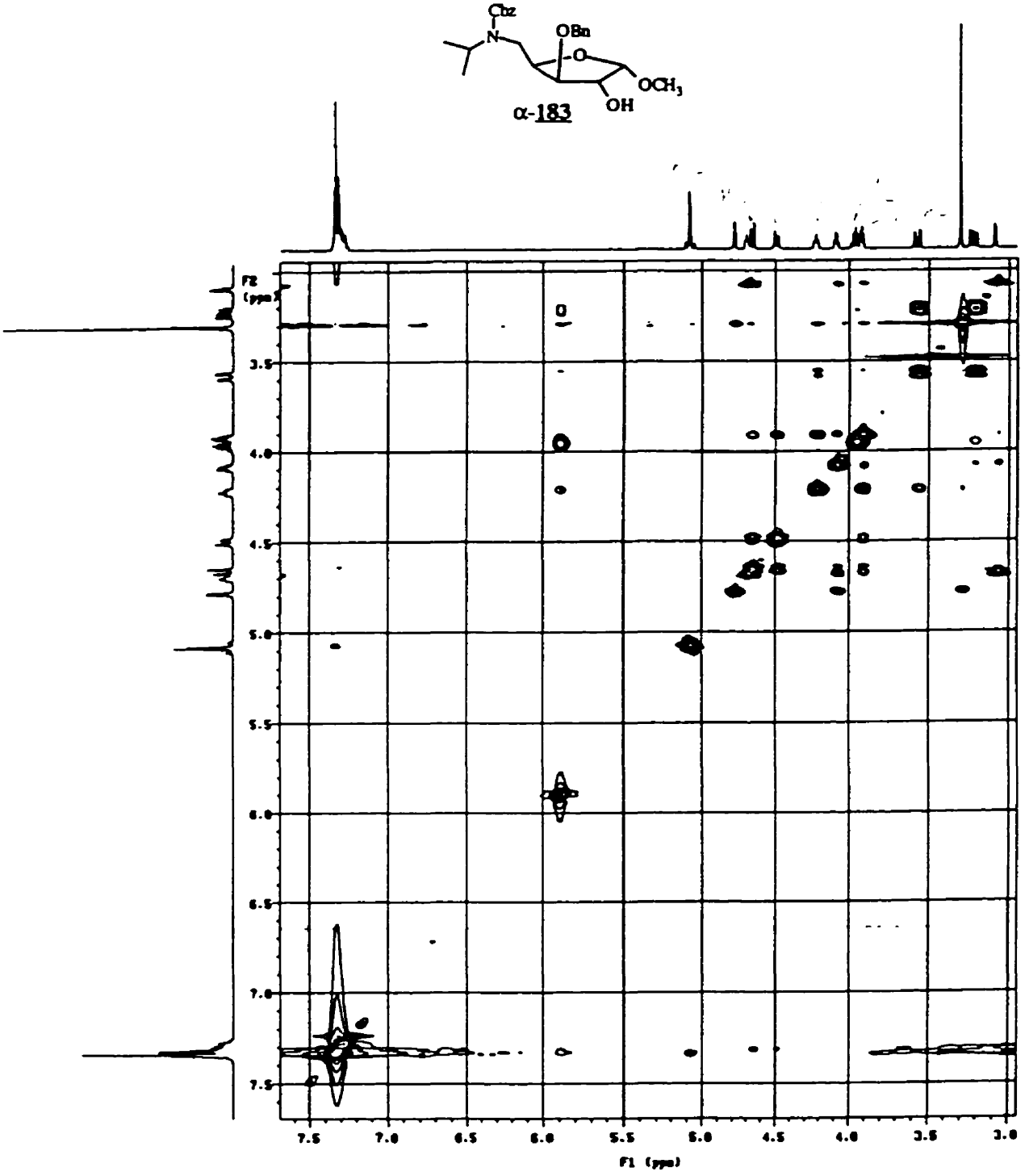
Appendix 13: ^{1}H NMR spectrum (200 MHz, CD_3SOCD_3, ambient temperature) of alcohol α-**183**



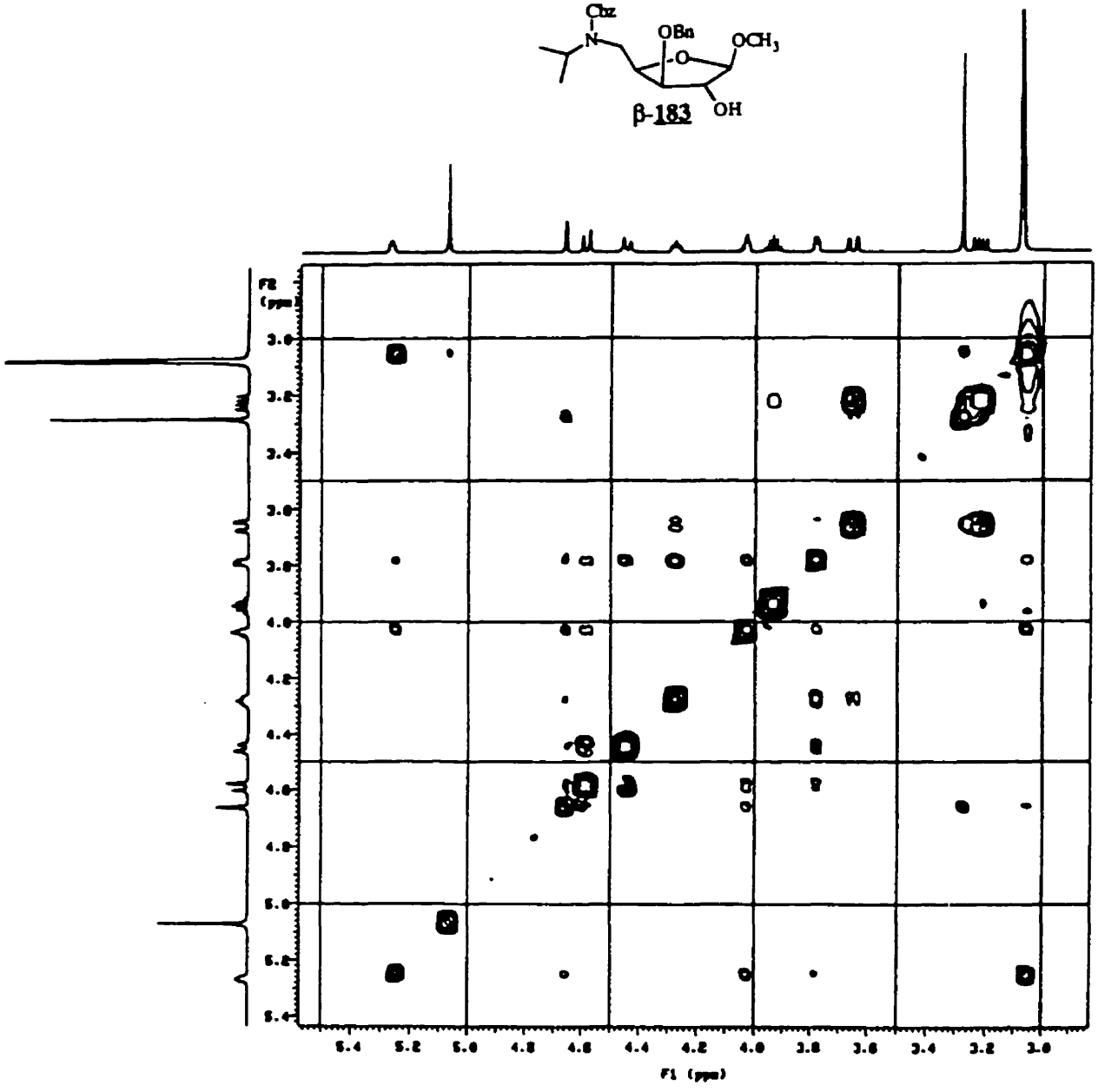
Appendix 14: ^{1}H NMR spectrum (500 MHz, CD_3SOCD_3, 90°C) of alcohol α-<u>183</u>



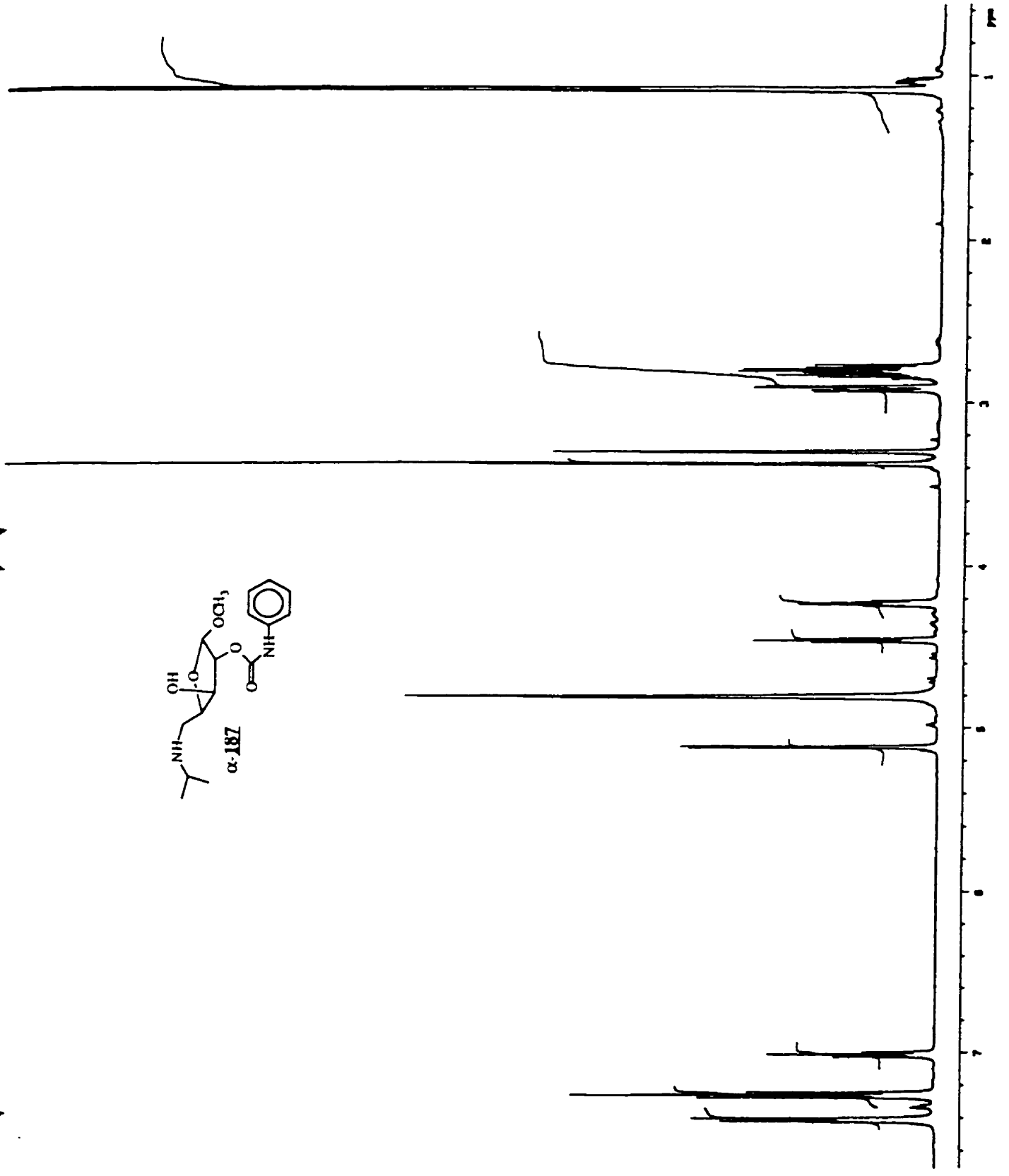
Appendix 15: 2D-NOESY map (500 MHz, CD_3SOCD_3, 90 °C, mix.=1440 msec.) of alcohol α-**183**



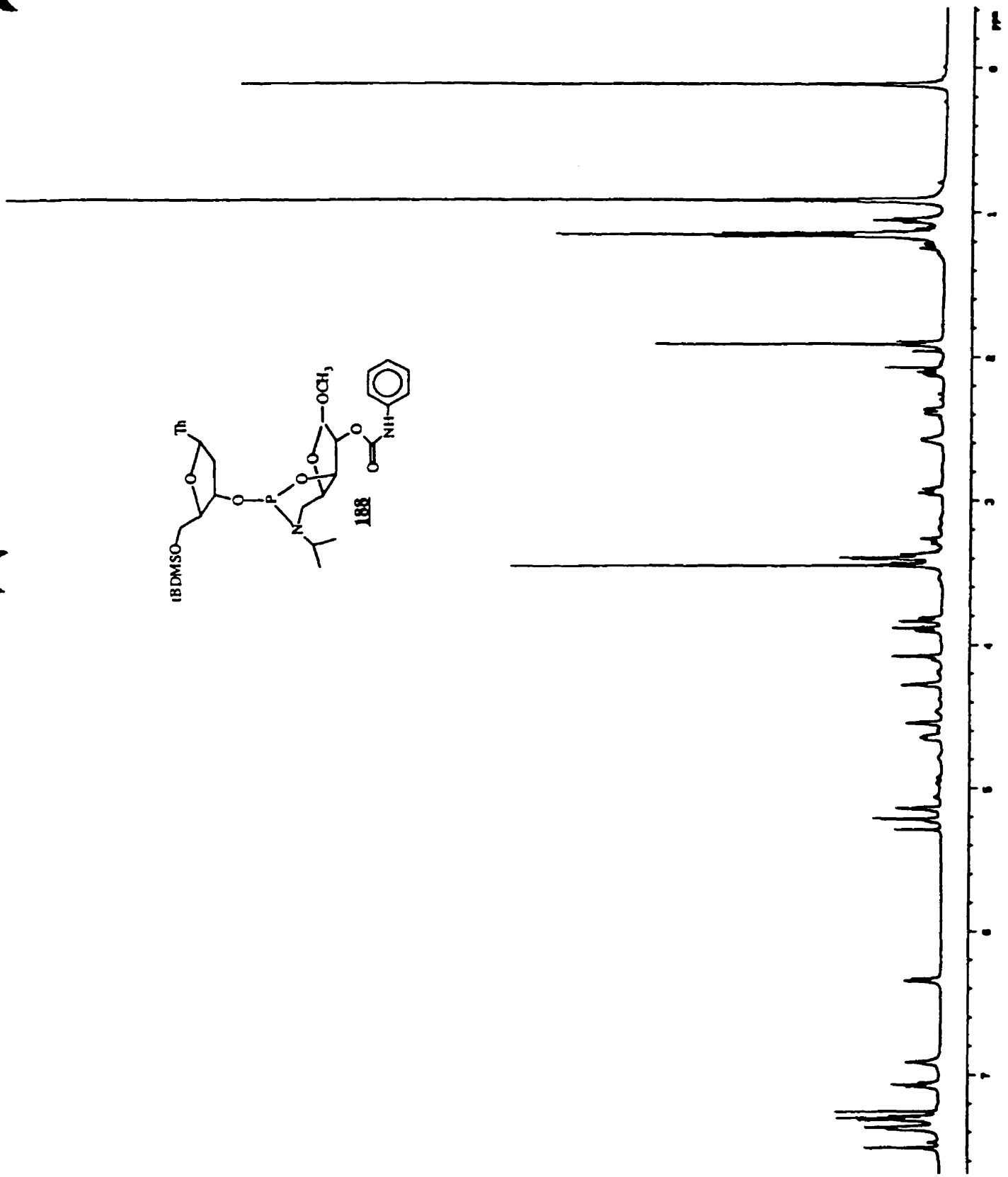
Appendix 16: 2D-NOESY map (500 MHz, CD_3SOCD_3, 90°C, mix. = 1120 msec.) of alcohol β-<u>183</u>



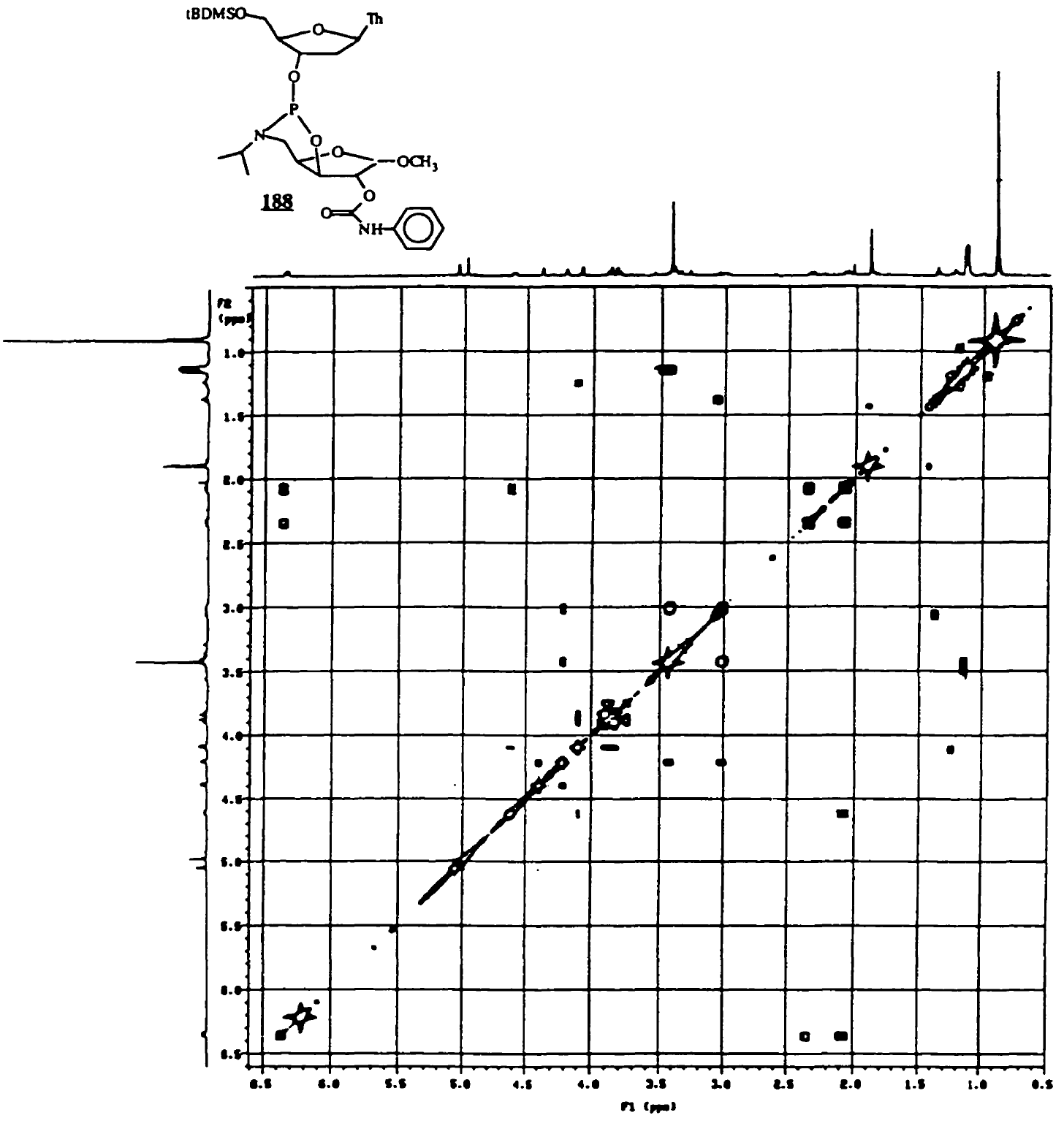
Appendix 17: ^{1}H NMR spectrum (500 MHz, CD_3OD) of γ-aminoalcohol α-**187**



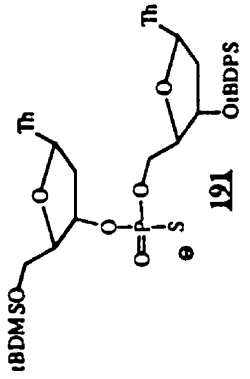
Appendix 18: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of oxazaphosphorinane **188**



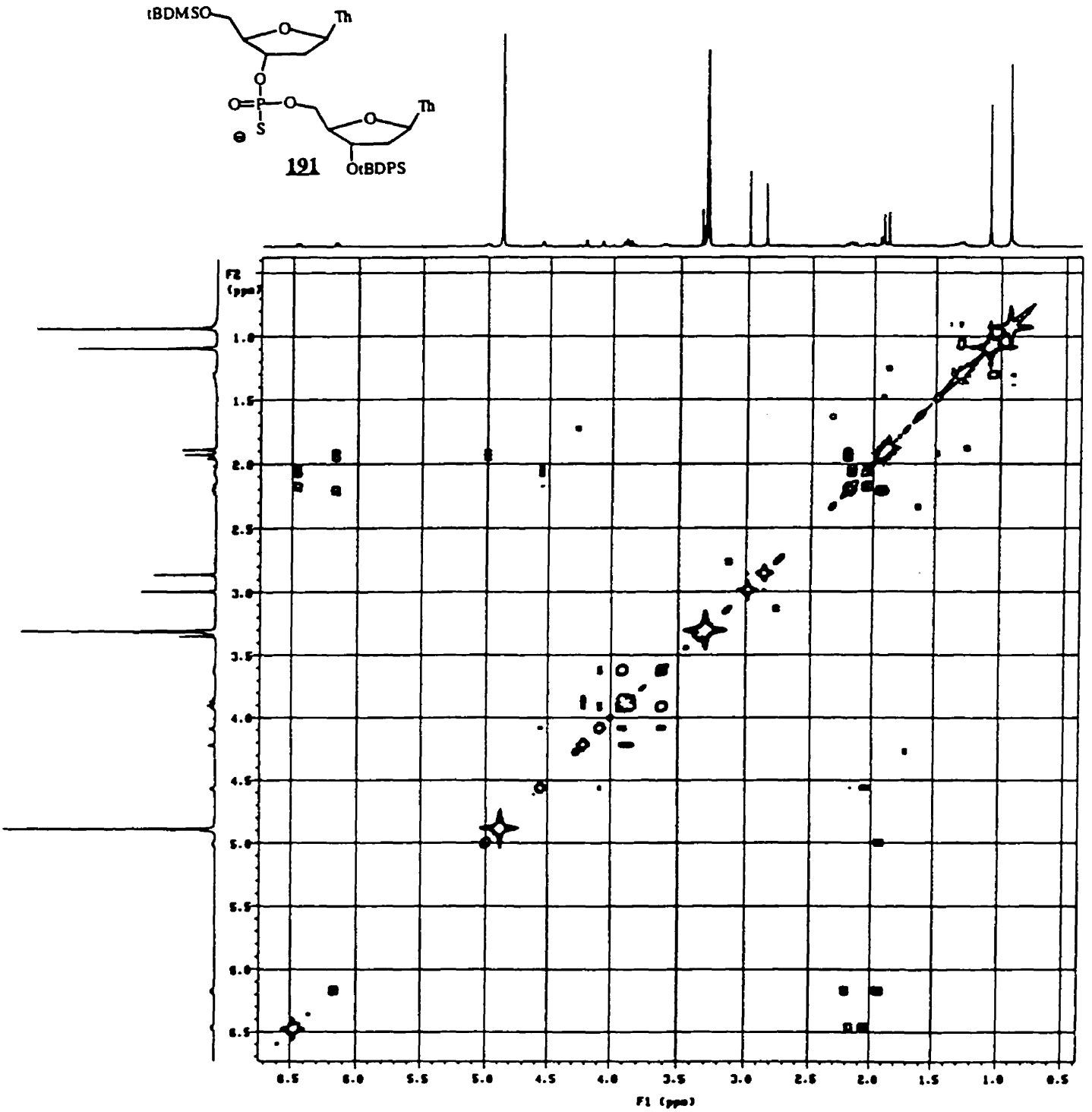
Appendix 19: 2D-COSY map (500 MHz, $CDCl_3$) of oxazaphosphorinane **188**



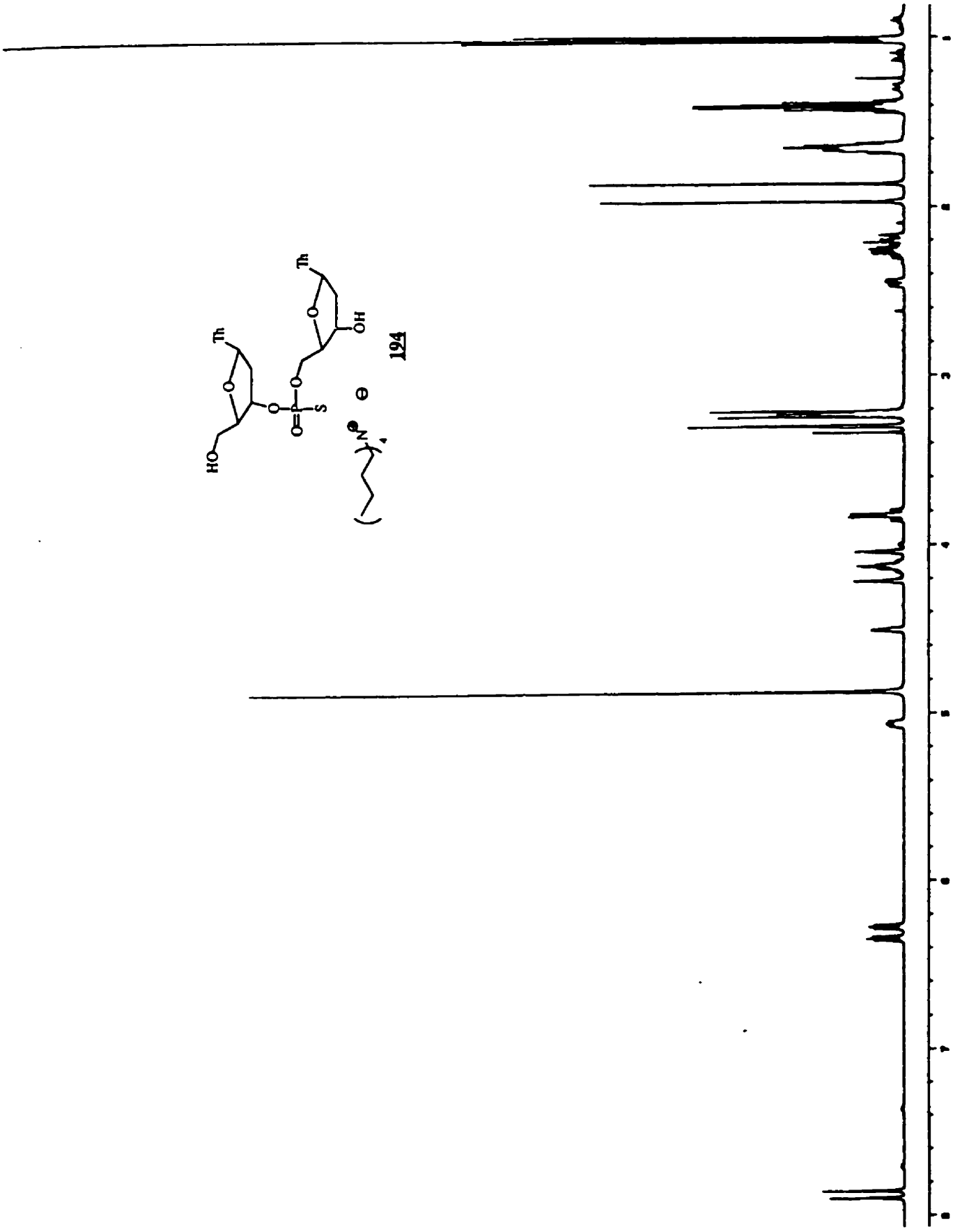
Appendix 20: ^{1}H NMR spectrum (500 MHz, CD_3OD) of dimer **191**



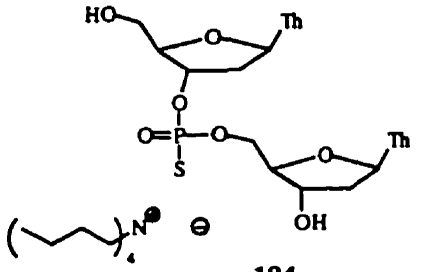
Appendix 21: 2D-COSY map (500 MHz, CD_3OD) of dimer **191**



Appendix 22: ^{1}H NMR (500 MHz, CD_3OD) spectrum of dimer **194**



Appendix 23: 2D-COSY map (500 MHz, CD_3OD) of dimer **194**



194

Appendix 24: 2D-HMQC map (500 MHz, CD_3OD) of dimer **194**

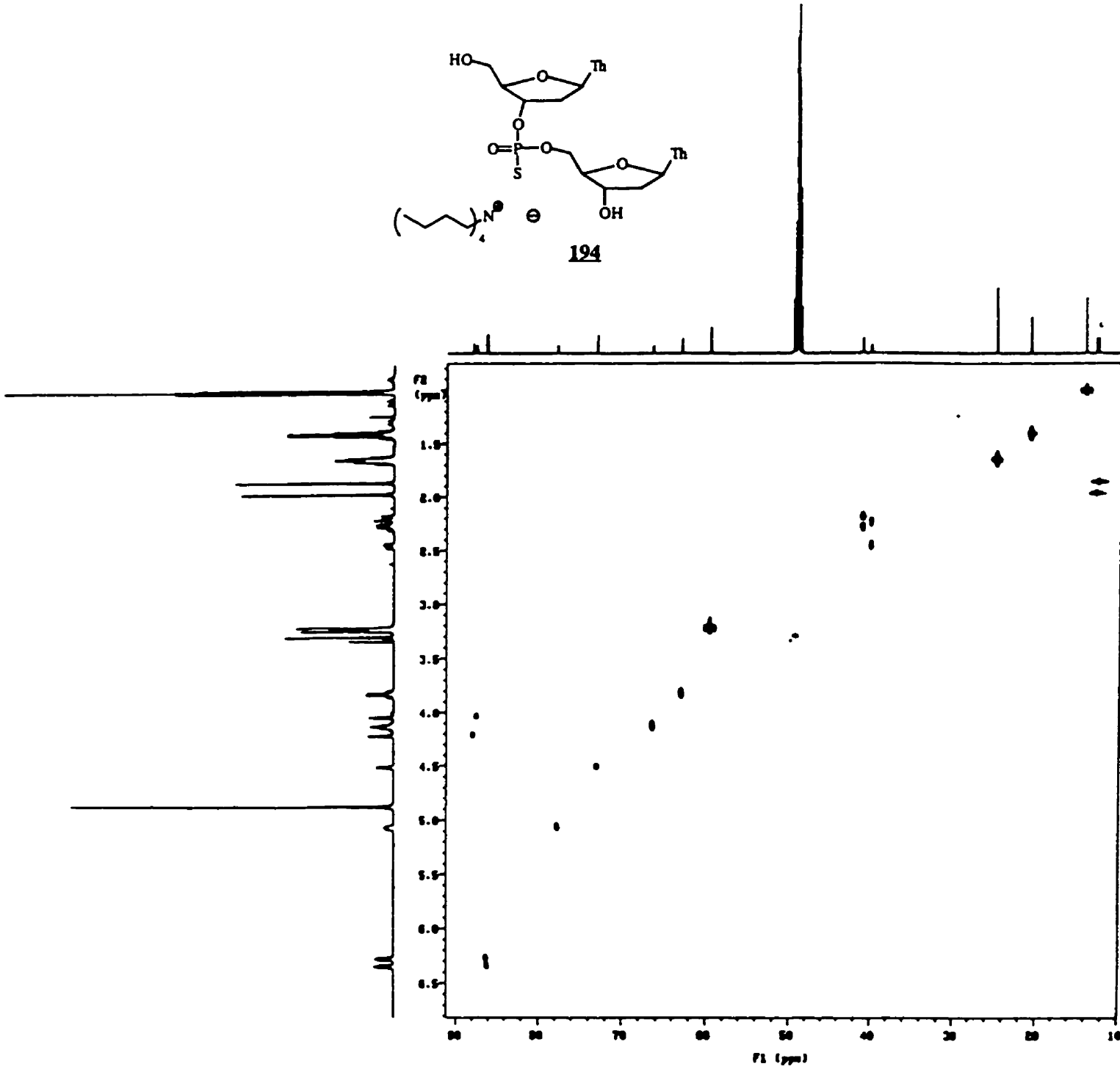


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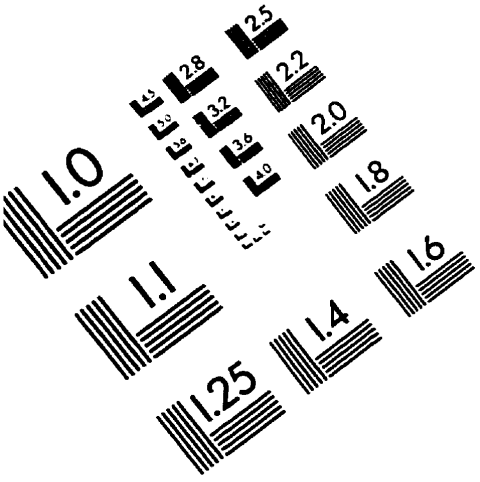
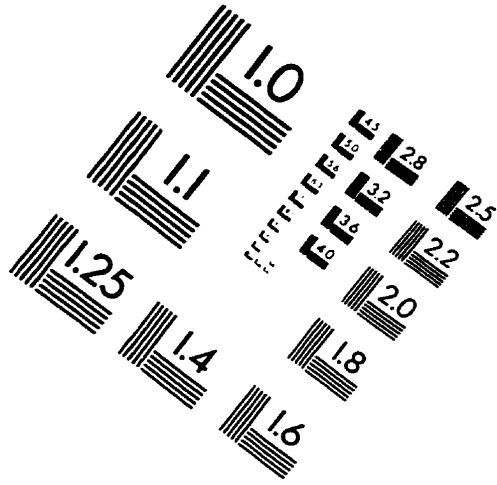
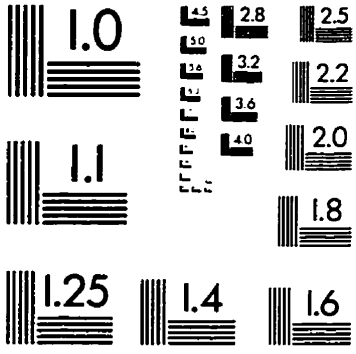
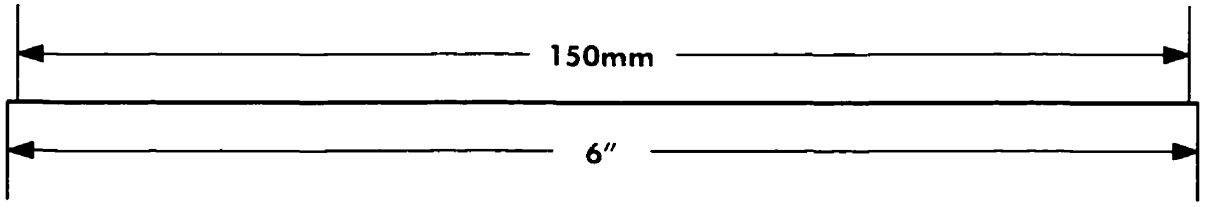
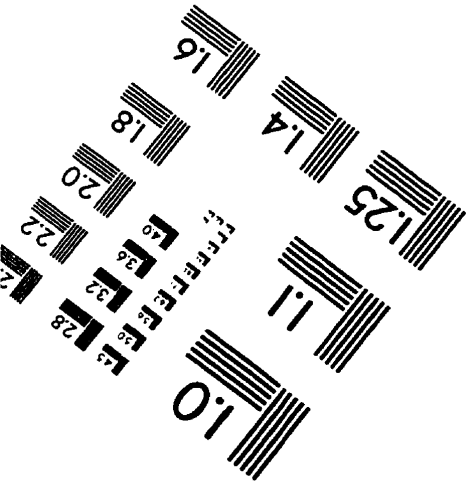
IMAGE EVALUATION
TEST TARGET (QA-3)

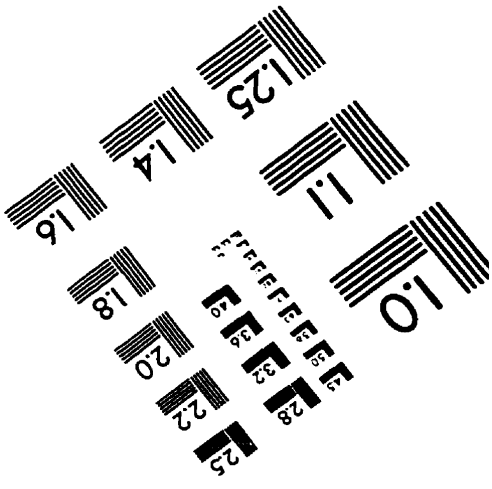












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