FLUORIDE ION IN THE SYNTHESIS OF NUCLEIC ACIDS: ACYLATION, ALKYLATION, TRANSESTERIFICATION AND DEPROTECTION

A Thesis

by

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FLUORIDE ION IN THE SYNTHESIS OF NUCLEIC ACIDS

To my wife, Diane

and my parents,

René and Thérèse Beaucage

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ABSTRACT

The use of the tert-butyldimethylsilyl protecting group was undertaken for the stepwise synthesis of polynucleotide triesters. The selective removal of the silyl protecting group was examined in order to promote the block synthesis of polynucleotides. The subsequent deprotection of mono- and dinucleotide triesters by fluoride ion in both aprotic and protic solvents was studied in detail.

A successful transesterification of triaryl and trialkyl phosphates or thiophosphates effected by cesium fluoride was applied to the synthesis of asymmetric nucleotide phospho-triesters. An interesting analog of double stranded DNA unit was synthesized by using this procedure.

An unusual fluoride assisted acylation of nucleosides was discovered along with a facile alkylation of nucleosides and mono- and dinucleotides.

ACYLATION, ALKYLATION, TRANSESTERIFICATION ET DEPROTECTION DES ACIDES NUCLEIQUES PAR L'UTILISATION DE L'ION FLUORURE

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Résumé

Le groupe protecteur tert-butyldiméthylsilyle fut utilisé dans la synthèse "par étape" des triesters polynucléotidiques. Le déblocage sélectif du groupe silyle fut examiné en vue de promouvoir la synthèse "par bloc" des polynucléotides. La déprotection subséquente des triesters mono- et dinucléotidiques par l'utilisation des fluorures en milieu protique et aprotique fut étudiée en détail.

La transestérification des phosphates et thiophosphates de triaryle et de trialkyle effectuée par le fluorure de césium fut appliquée à la synthèse asymmétrique des triesters mononucléotidiques. Une unité d'un analogue intéressant d'une double chaîne d'ADN fut synthétisée selon cette méthode.

Une acylation peu commune des nucléosides, ainsi qu'une alkylation facile des nucléosides et des mono- et dinucléotides, assistées par l'ion fluorure, furent découvertes.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

T	Deoxythymidine
dC	Deoxycytidine
dA	Deoxyadenosine
dG	Deoxyguanosine
cTMP	Deoxythymidine-(3'→5')cyclic phosphate
Thy	1-(Thyminyl)
βCEP	β-Cyanoethyl phosphate
TPS	2,4,6-Triisopropylbenzesulfonyl chloride
TPSOH	2,4,6-Triisopropylbenzesulfonic acid
Pyr	Pyridine
mmt	p-monomethoxytrityl
dmt	p,p'-dimethoxytrityl
tr	Trityl
BzCl	Benzoyl chloride
Bz	Benzoyl
Pv	Pivaloyl
Pv_2O	Pivalic anhydride
Ac	Acetyl
Myr	Myristoyl
BSA	bis-Trimethylsilyl acetamide
TBDMSCl	tert-Butyldimethylsilyl chloride
TBDMS	tert-Butyldimethylsilyl
TIPS	Triisopropylsilyl

TMS	Tetramethylsilyl
CE	Cyanoethyl
TCE	2,2,2-Trichloroethyl
ϕ	Phenyl
TCEOH	2,2,2-Trichloroethanol
ϕOH	Phenol
i-PrOH	iso-Propanol
TBAF	tetra-n-Butylammonium fluoride
CsF	Cesium fluoride
PMR	^{1}H Nuclear Magnetic Resonance
CMR	^{13}C Nuclear Magnetic Resonance
g.l.c.	Gas-liquid chromatography
R.T.	Room temperature
MS	Mesitylenesulfonyl Chloride

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INTRODUCTION

In an attempt to elucidate the mechanism of protein biosynthesis, the operon theory was introduced by Jacob and Monod in the early sixties[1]. A decade after, Khorana and his coworkers[2] reported the total synthesis of the structural gene for an alanine transfer RNA from yeast. Although many aspects of transcription[3,4] and DNA enzymology[5,6] were studied, this 77-nucleotide long DNA was found occasionally unsuitable for studies of certain problems of biochemical interest. Recently, the Khorana group[7] reported as a complementary work, the total synthesis of the structural gene for the precursor of a Tyrosine suppressor transfer RNA from Escherichia coli. They have many practical reasons for the choice of that gene; one of them is the accuracy of the nucleotide sequence of the corresponding tRNA which has been determined independently by two groups of workers[8,9]. The ultimate goal of this gene synthesis is to effect a controlled transcription of the latter into the functional tyrosine tRNA. Elucidation of the promoter sequence as well as the precise determination of the lengths of the DNA regions involved in the initiation and termination of transcription will therefore be required in order to understand the biological signals for transcription.

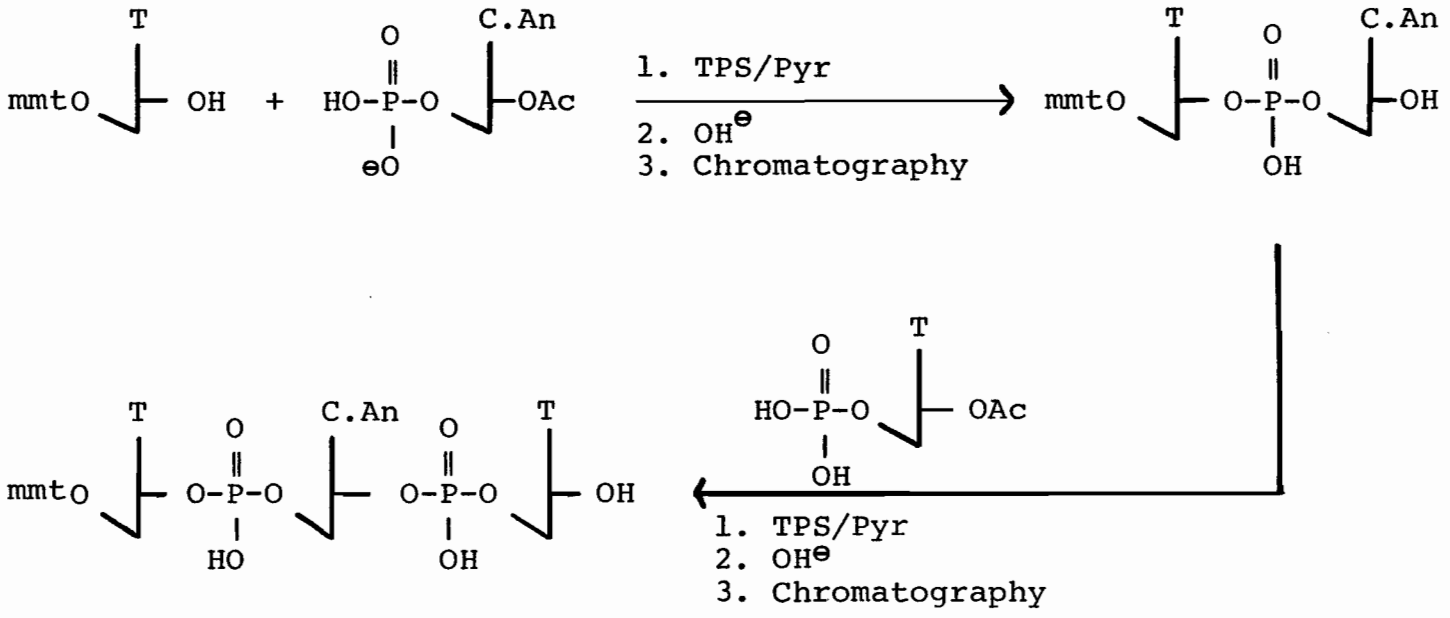
In the same vein, Narang's[10,11] and Caruthers's groups[12,13] reported the chemical and enzymatic synthesis of the Lactose

operator of Escherichia coli. Caruthers and his coworkers[14] found how lac repressor recognizes lac operator, and the mechanism of action of the lac promoter is presently under intensive investigation. These studies require therefore the availability of various deoxyribonucleotides of defined and specific sequences. The efficiency of nucleic acid synthesis becomes of prime importance.

Khorana's technology is concerned with the so called "diester approach". This technique involves the condensation of a nucleoside suitably protected at the 5'-position by an acid labile p-monomethoxytrityl group, with a nucleoside bearing at the 5'-position a phosphate group and an alkali labile protecting group at the 3'-position. When required, the free amino group of certain bases must be adequately protected prior to the condensation. The formation of the natural 3'→5' link is induced by the addition of a phosphate activator which is usually an arenesulfonyl chloride such as mesitylene sulfonyl chloride (MS) or triisopropylbenzene-sulfonyl chloride (TPS). Scheme I illustrates the stepwise synthesis of a di- and trinucleotide. The method consists in successive condensation of a growing oligonucleotide chain with a mononucleotide.

This approach is generally used for syntheses up to tri- and tetranucleotides[15-19] where yields vary between 40-95%. However, this approach presents some disadvantages. The yield

Scheme I



TPS = 2,4,6-triisopropylbenzesulfonyl chloride

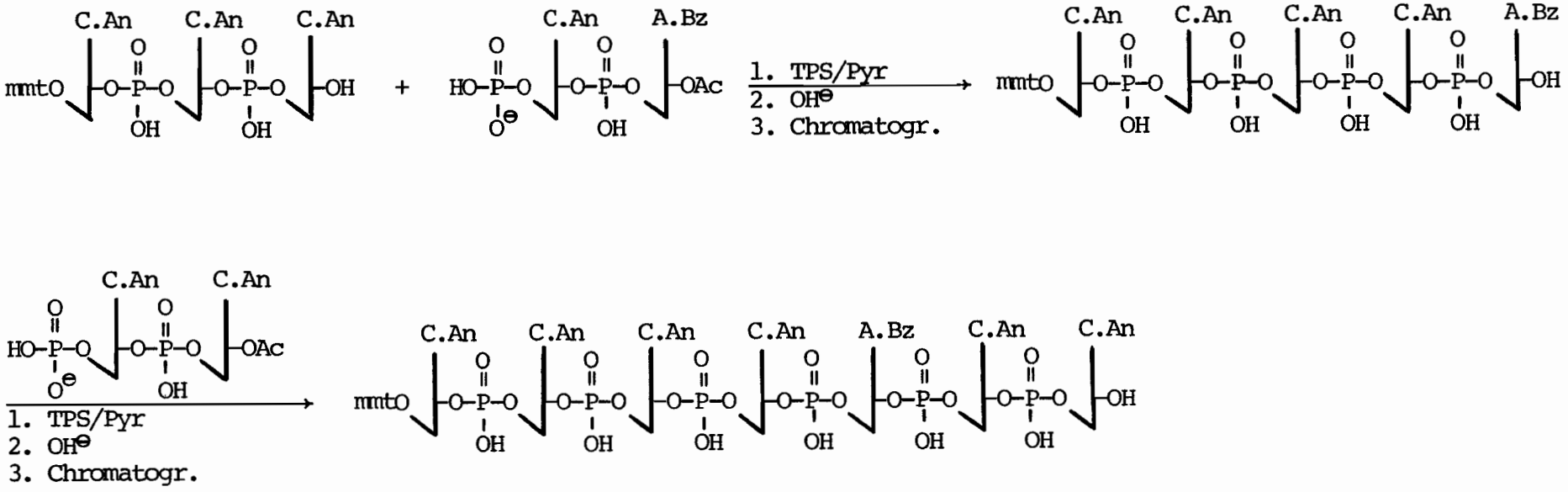
mmt = p-monomethoxytrityl

An = p-anisoyl

of coupling decreases with increasing chain length and the separation of the product from the starting oligonucleotide is often difficult because the molecular weight of the product and the starting material differ only by one nucleotide. The purification problems combined with the fact that the chain is growing by only one nucleotide at the time, make this approach highly time consuming. Most of the problems are overcome by linking preformed oligonucleotide blocks. Scheme II exemplifies the blockwise synthesis of segments of the tyrosine suppressor tRNA precursor gene[20].

The reaction between blocks has been shown to go at a slow rate and in lower overall yields with respect to the stepwise condensation. However, the yields can be improved by using an increasingly large excess of the nucleotide component with elongation of the chain. The synthesis of a hexadecanucleotide was achieved via the blockwise addition of tetranucleotide units[21]. Longer chains can be made when a template complementary to the blocks to be connected is available, as well as the enzyme DNA ligase, which is used to effect the linkage[22-26]. Despite the fact that the block-wise approach has greatly improved the rapidity for chain elongation, the separation of the starting material and the nucleotidic product is still a time consuming process. Considerable efforts were applied in the discovery of new tools and faster separation techniques for the isolation and

Scheme II



Bz = benzoyl.

characterization of oligonucleotides. Koster and Kaiser[27] used alcohols or mixtures of alcohols with ionic strength gradients for elution of oligonucleotides for DEAE-cellulose[28]. Narang and Michniewicz[29] employed Avicel-cellulose thin layer chromatography for the separation and identification of mixtures of oligothymidylates as well as mononucleosides and nucleotides. A successful separation of oligoribo- and oligodeoxyribonucleotides was obtained by reverse phase chromatography on columns of polychlorotrifluoroethylene support coated with methyltrialkylammonium chloride using ammonium acetate eluents[30,31]. Purification based on the varied affinities between a mixture of oligonucleotides and nucleotide chains of specific length and sequence immobilized on a cellulose column has been investigated[32-36] and it has been reported that a mixture of hexa-, hepta-, octa-, and nonaadenylic acids was successfully resolved by a column of cellulose-d$(pT)_9$. Finally, the application of high-performance liquid chromatography (HPLC) for the separation of oligodeoxyribonucleotides of intermediate size has been reported by Cook[37]. Long chain synthesis by the "diester approach" without the aid of DNA ligase for coupling procedure are still restricted despite the progress in isolation techniques. The phosphodiester function has been shown to undergo activation under certain conditions resulting in the formation of undesirable side products[38], which increase in probability

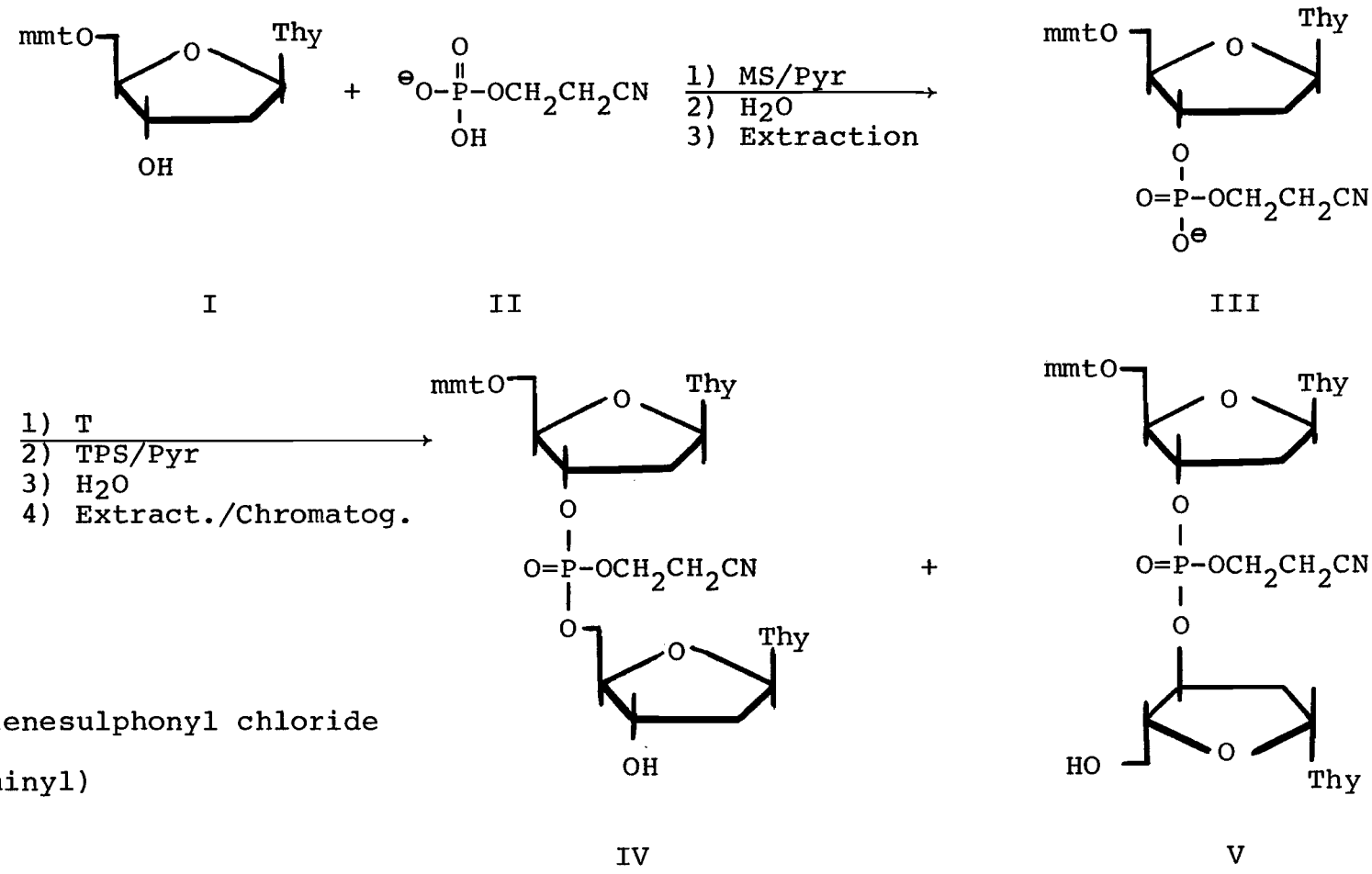
with increasing chain lengths.

Inhibition of the formation of such by-products can be effected by protecting the phosphate monoester via esterification. This strategy is the basis for the so called "triester approach". Michelson and Todd[39] were the first to use this approach in the synthesis of a benzyl dinucleoside phosphate. The phosphotriester was not isolated but directly converted to the diester via hydrolysis with hot aqueous hydrochloric acid. A decade was required for the triester approach to begin its ascension. The pioneering work by Letsinger[40] and Eckstein[41] lay the foundation for this productive approach as illustrated in Scheme III.

The side product V is eliminated when a suitably protected nucleoside such as 3'-O-acetyl thymidine is used in the coupling reaction procedure[41]. By the triester approach it was then possible to synthesize large scale nucleotidic material which was easily purified on silica gel in a minimum of time.

The search for stable phosphate protecting groups as well as for methods for their removal was intensively pursued. Khorana[42-44] initially used the 2-cyanoethyl group for terminal phosphate protection. The same protecting group was successfully used as we have seen in Scheme III by Letsinger[40,45-47], Ogilvie[48-51] and Smrt[53,54]. The reagents for introducing the protection are readily available and the deblocking can be readily achieved under mild

Scheme III[40]



MS = mesitylenesulphonyl chloride

Thy = 1-(thyminyl)

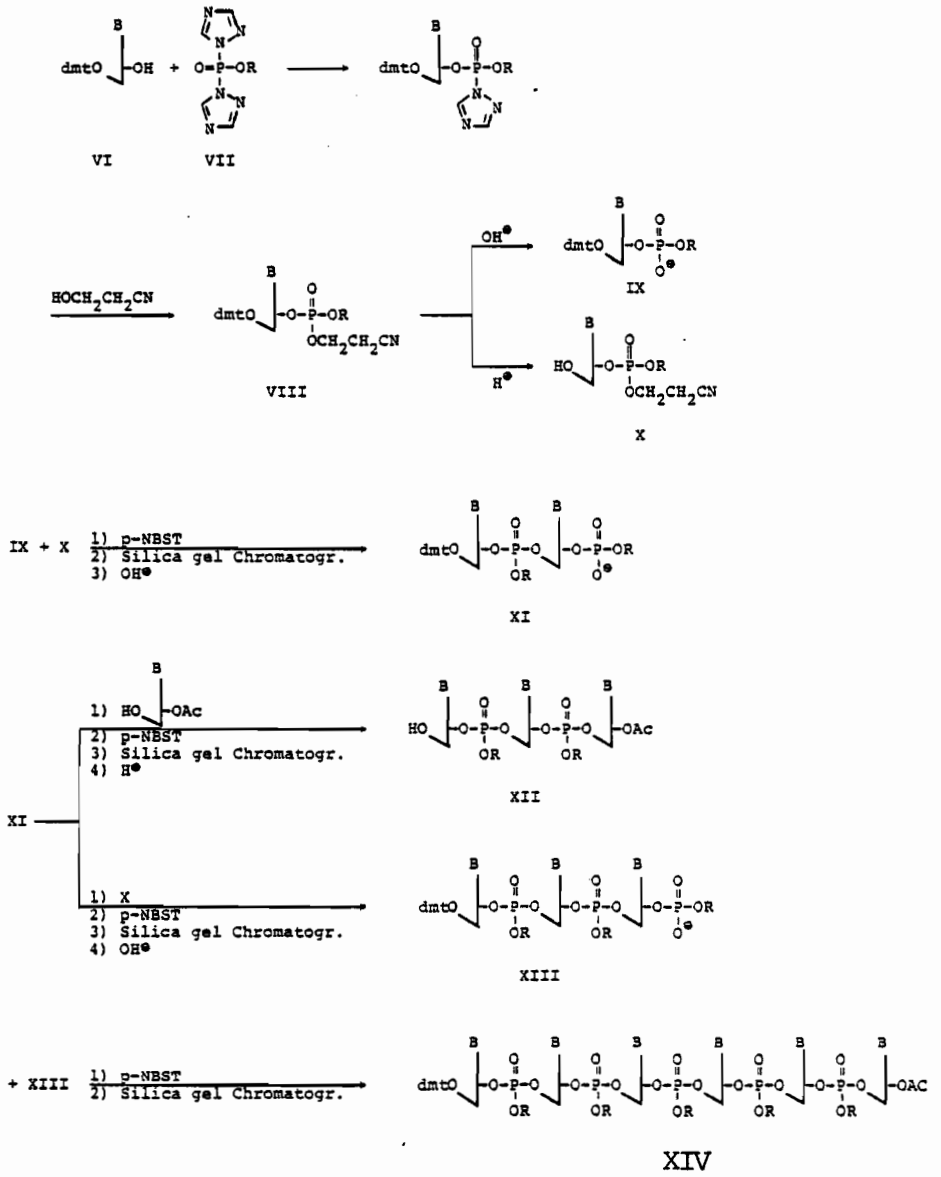
basic conditions. The trichloroethyl group, which was originally introduced by Woodward[52] for the synthesis of cephalosporin, was first introduced to phosphate protection by Eckstein[41,55,56]. The protecting group was extensively used by Neilson[57-59] in oligoribonucleotide synthesis. Being much more stable than 2-cyanoethyl group toward work-up and basic conditions, the main disadvantage of the trichloroethyl group lies in its removal. The use of zinc dust in 80% acetic acid[41] or copper-zinc couple in N,N-dimethylformamide[56] as well as zinc dust in pyridine/acetic acid mixtures[56] often gave quite low yields[60]. Recently, Wiewiorowski and coworkers[61] claimed that the trichloroethyl group can be removed on oligoribonucleotides by using zinc powder in acetylacetone/ anhydrous pyridine solution. Apparently, the removal of the trichloroethyl group occurred within 10 min in yields greater than 90%. It is worthwhile to mention also that phenyl[62-65], o-chlorophenyl[63,65] and p-methylthiophenyl[65] phosphate protecting groups were used successfully in the synthesis of oligonucleotides. Furthermore, in order to apply the block synthesis to the triester approach, several strategies were developed. Narang[10,66] introduced elegantly the blockwise approach to the chemical synthesis of the Lactose operator via the phosphotriester method. The appropriate nucleoside (VI) was treated with a new phosphorylating agent (VII) and with 2-cyanoethanol affording the triester VIII which is

the key intermediate. This is illustrated in Scheme IV.

Analogously, van Boom and his coworkers reported the use of 2,2,2-trichloroethyl-2-chlorophenyl phosphorochloridate (XV) as a convenient reagent for the formation of internucleotide linkages[67-69]. The reaction of XV with a suitably protected nucleoside (XVI) produced the triester XVII. The trichloroethyl phosphate protecting group was then removed by treatment with zinc dust and triisopropylbenzesulfonic acid (TPSOH) in pyridine for 3 min. The resulting diester (XVIII) was coupled with the nucleoside XIX affording the dinucleotide XX. Selective deblocking at both ends of XX gave the key intermediates (XXI) and (XXII) for a blockwise synthesis as illustrated by Scheme V.

Recently, Ramirez and his collaborators[70,71] described the application of cyclic enediol pyrophosphate (XXIII) to the synthesis of deoxyribonucleotides. The idea consists of the reaction of 5'-O-p-monomethoxytritylthymidine (XXIV) with XXIII in the presence of triethylamine. The resulting triester (XXV) was further condensed with thymidine under similar conditions. The fully protected dinucleotide (XXVI) was isolated in 82% yield. After the removal of the p-monomethoxytrityl group with trifluoroacetic acid, the acetoinyl phosphate protecting group was removed under mild basic conditions (aqueous Et_3N) affording the natural thymidilyl-(3'→5')-thymidine (XXVII) (Scheme VI).

Scheme IV

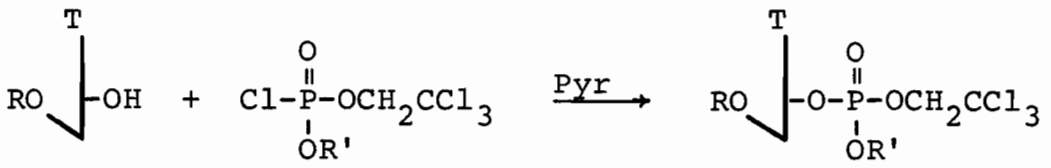


B = thymine
N-benzoyladenine
N-benzoylcytosine
N-acetylguanine

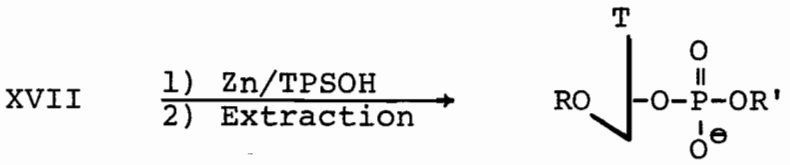
dmt = p-dimethoxytrityl
R = p-chlorophenyl
p-NBST = p-nitrobenzesulfonyltriazolide

Note: Anhydrous solution of triethylamine in pyridine was used for the quantitative removal of the cyanoethyl group, while N-acyl and p-chlorophenyl groups were untouched (A.K. Sood and S.A. Narang, Nucleic Acids Res., 4(8), 2757 (1977)).

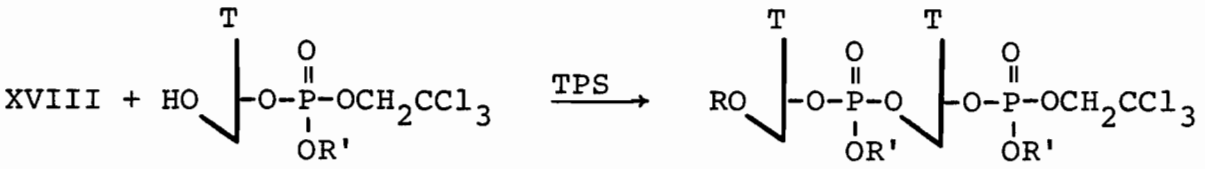
Scheme V



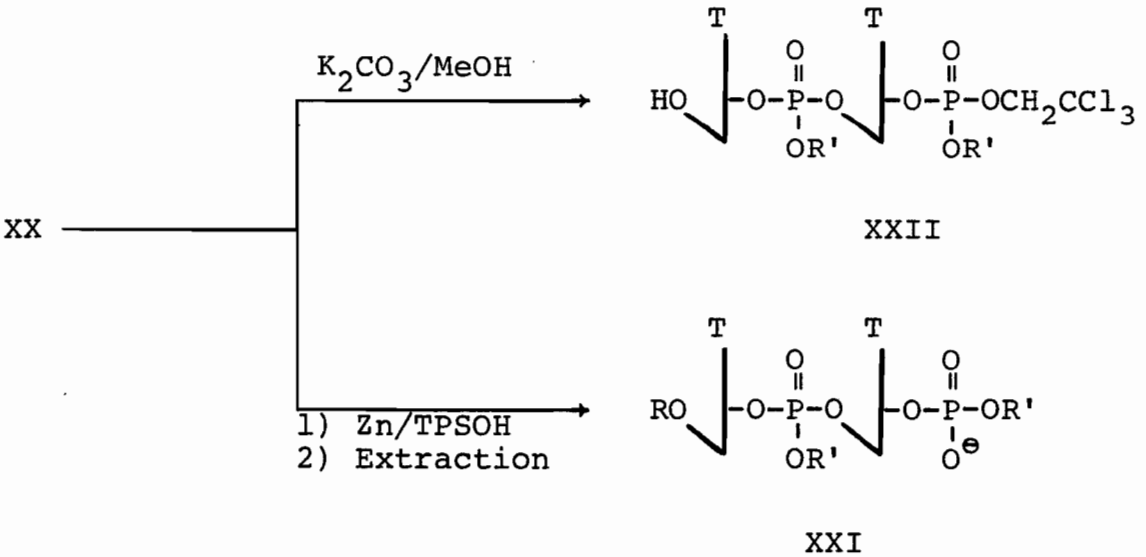
XVI XV XVII



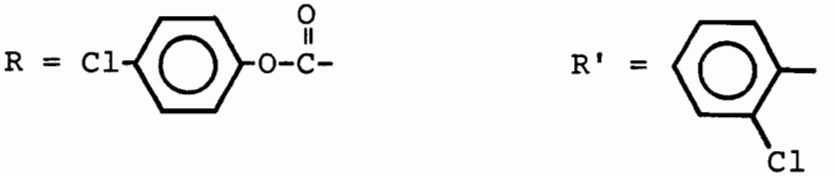
XVIII



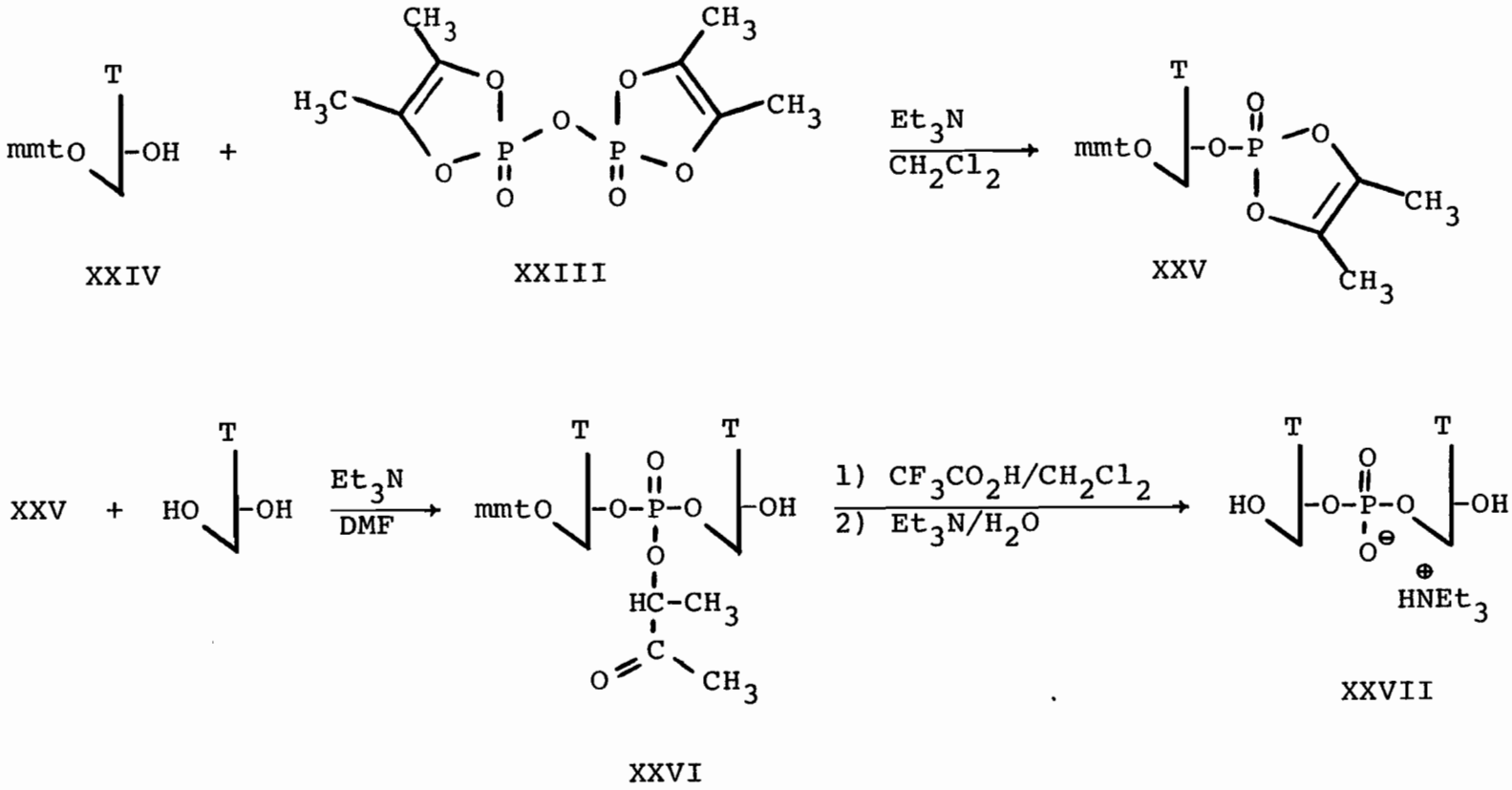
XIX XX



XXII XXI



Scheme VI


mmtO
T
OH
+
CH3
H3C
O
P
Et3N
CH2Cl2
XXIV
XXIII
XXV
XXV + HO OH
DMF
1) CF3CO2H/CH2Cl2
2) Et3N/H2O
HC-CH3
C
CH3
HNEt3
XXVI
XXVII

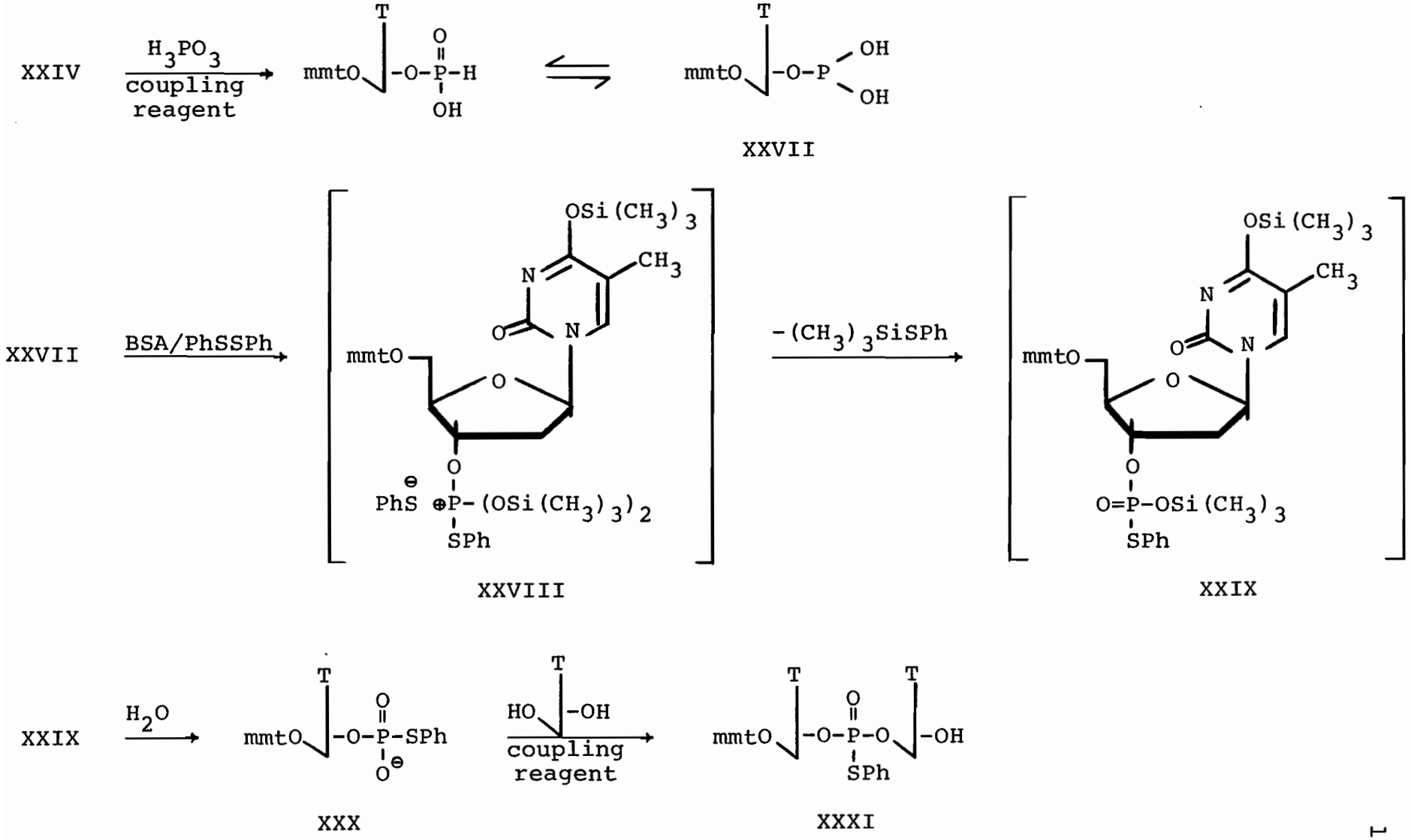
According to this procedure, a maximum of 2% of the unnatural 3'→3' isomer was produced and less than 2% of (3'→5') internucleotide bond cleavage occurred in the deprotection step. A stepwise synthesis of a tetranucleotide was also reported[71].

Hata and his coworkers[72-74] suggested the phenylthio group as a phosphate protecting group in oligonucleotide synthesis. Their approach involved the condensation of 5'-O-p-monomethoxytritylthymidine (XXIV) with phosphorous acid in the presence of a coupling reagent (arylsulfonylamides or chlorides). The phosphite XXVII was then reacted with bis-trimethylsilyl acetamide (BSA) in the presence of diphenyl disulfide. The intermediates XXVIII and XXIX were presumably formed, which upon aqueous work-up gave the diester XXX. The coupling of the latter with thymidine gives the triester XXXI as exemplified by Scheme VII.

A trinucleotide was synthesized according to the latter procedure in 40% yield.

The S-ethyl group, as a phosphate masking group, has been used in nucleotide synthesis by Cook[75], and Nussbaum and his collaborators[76-80]. The stability of the group to a variety of reaction conditions as well as its susceptibility to nucleophilic displacements after oxidation with iodine make the S-ethyl phosphorothioate moiety an attractive protecting group. Recently, Takaku[81] reported that 8-quinolyl dihydrogen

Scheme VII

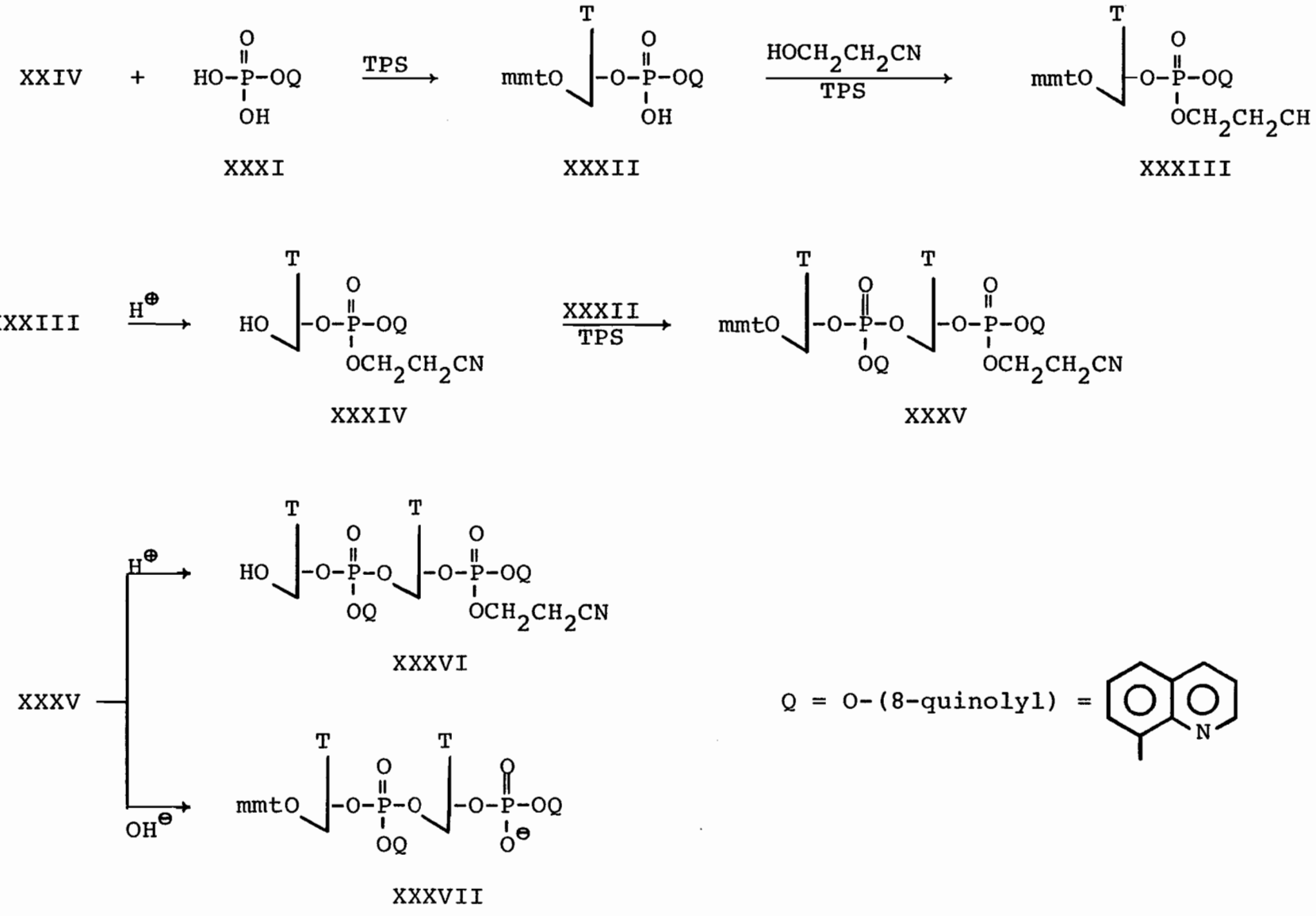

XXIV
H_3PO_3
coupling reagent
mmtO
T
O
P
H
OH
XXVII
BSA/PhSSPh
$OSi(CH_3)_3$
CH_3
N
O
PhS
$P-(OSi(CH_3)_3)_2$
SPh
XXVIII
$-(CH_3)_3SiSPh$
$O=P-OSi(CH_3)_3$
XXIX
H_2O
$O^{\ominus}$
XXX
HO
OH
coupling reagent
XXXI

phosphate has been used successfully as a phosphorylating agent. Shortly after, Hata's group introduced the 8-quinolyl nucleoside 5'-phosphate as a useful intermediate for the synthesis of nucleoside 5'-di- and 5'-tri-phosphates[82] as well as the preparation of nucleoside 3'-5'-cyclic phosphates[83]. Meanwhile, the 8-quinolyl phosphate protecting group was adapted to the phosphotriester approach[84,85] in the following manner: the nucleoside XXIV was treated with 8-quinolyl dihydrogen phosphate (XXXI) in the presence of TPS. The resulting diester (XXXII) was then condensed with 2-cyanoethanol in the presence of TPS affording the triester XXXIII, which by selective deprotection with base or acid and subsequent TPS assisted condensation gave the adequate blocks (XXXVI and XXXVII) for a tetranucleotide synthesis (Scheme VIII).

It is noteworthy to mention that van Tamelen[86] used the methyl group as a phosphate protecting group in oligoribonucleotide synthesis. For the deprotection of the methylphosphotriester intermediate, thiophenoxide ion generated in <u>situ</u> (C_6H_5SH in Et_3N/dioxane) was used. The demethylation is apparently clean and usually proceeds in high yield. Therefore, the methyl group as a phosphate protecting group becomes of interest, being of small size, stable and apparently easily removed.

Reese and his coworkers[87] proposed 4-nitrophenyl phenylphosphorochloridate (XXXVIII) as a phosphorylating agent for

Scheme VIII

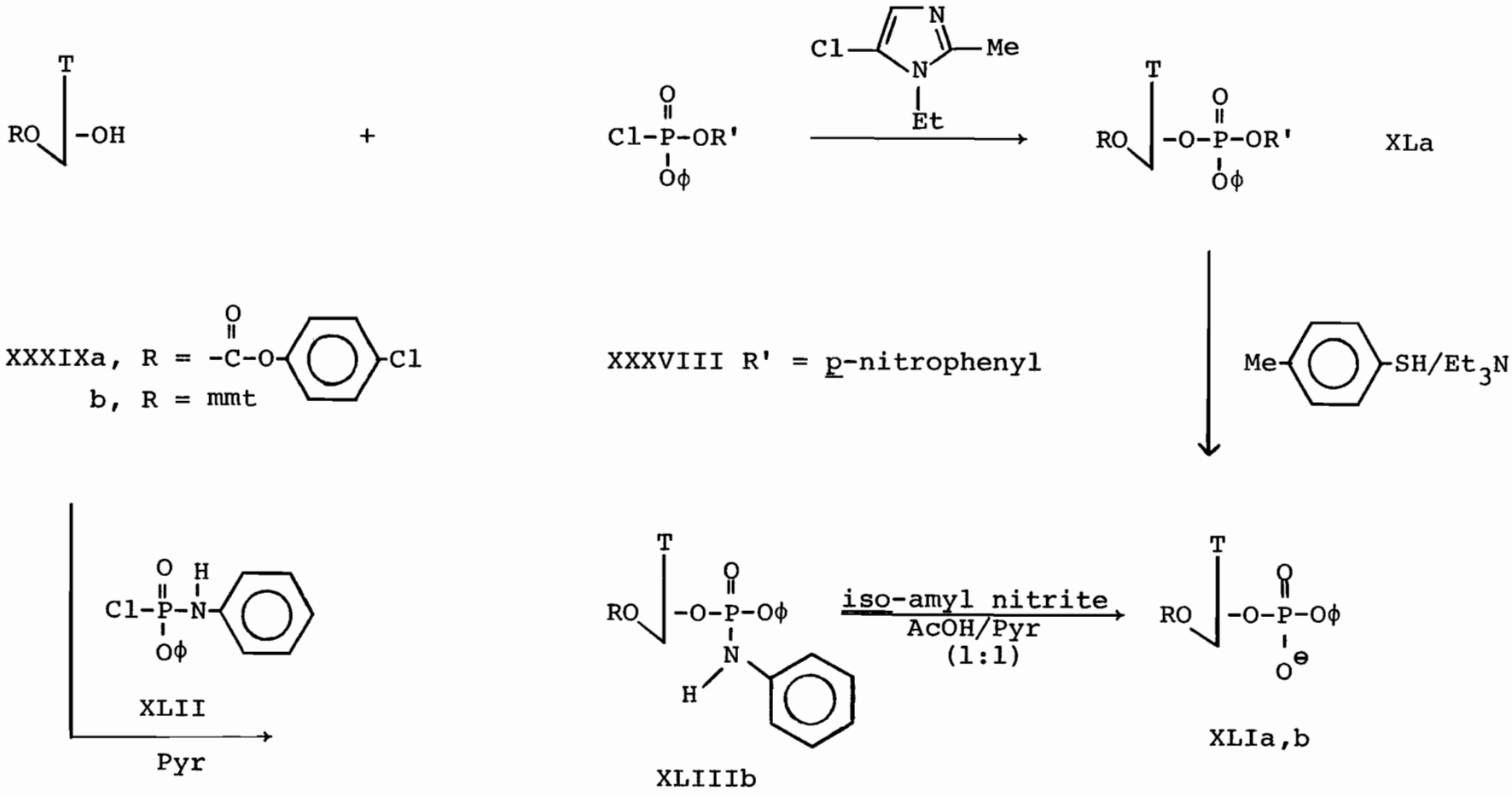

XXIV
TPS
XXXI
XXXII
HOCH2CH2CN
TPS
XXXIII
H⊕
XXXIV
XXXII
TPS
XXXV
H⊕
XXXVI
XXXV
OH⊖
XXXVII
Q = O-(8-quinolyl) =
N

oligonucleotide synthesis. The triester XLa formed from the reaction of XXXVIII with a suitably protected nucleoside XXXIXa was deprotected by p-thiocresol and Et_3N in acetonitrile giving the diester XLIa in quantitative yield. XLI is therefore readily available for oligonucleotide synthesis (Scheme IX). Phenyl N-phenylphosphoroamidochloridate (XLII) was also reported as a new phosphorylating agent[88]. The triester XLIIIb obtained from a reaction pattern analogous to that described previously was deprotected almost quantitatively with iso-amyl nitrite[89] in acetic acid/pyridine (1:1) to the diester XLIb.

Several aryl and aralkyl protecting groups including 2-arylmercaptoethyl[90,91], benzhydryl[92], α-pyridylethyl[93], fluorene-9-methyl[94], 2,4-dinitrophenyl[95,96], 4-chloro-2-nitrophenyl[97], 1-oxido-pyridylmethyl[98] and the o-nitrobenzyl[99] moieties have been studied. The latter have the advantage of photolability under conditions which do not affect the purine or pyrimidine bases.

An important aspect of oligonucleotide synthesis is the choice of the protecting groups for the hydroxyls of nucleoside moieties as well as the amino groups of heterocyclic bases. The selection of the protecting groups must be compatible with a given phosphate protecting group if the triester approach has been chosen for the synthesis. During stepwise or blockwise elongation of the chain and depending

Scheme IX


T
RO
-OH
+
Cl
N
Me
N
Et
Cl-P-OR'
Oϕ
T
RO
-O-P-OR'
Oϕ
XLa
XXXIXa, R = -C-O-Cl
b, R = mmt
XXXVIII R' = p-nitrophenyl
Me-SH/Et3N
Cl-P-N-H
Oϕ
XLII
Pyr
RO
-O-P-Oϕ
N
H
XLIIIb
iso-amyl nitrite
AcOH/Pyr
(1:1)
RO
-O-P-Oϕ
Oe
XLIa,b

at which end the chain is growing, the selective removal of the protecting group at the 5'-position or 3'-position is desirable. For this purpose, the 5'-hydroxy function due to its different reactivity with respect to a secondary alcohol can be selectively blocked by the triphenylmethyl group[100] and its derivatives[101,102]. The introduction of each p-methoxy group into the phenyl ring makes the protecting group 10 times easier to be removed, but, unfortunately increases the possibility for reaction with the secondary 3'-hydroxy function. Nevertheless, the 5'-hydroxy group can be protected selectively by the p-monomethoxytrityl group in good yield. The removal of the protecting group is effected by 80% aqueous acetic acid or by pyridine-acetic acid buffer[21,103] at room temperature.

Recently, Letsinger and his collaborators[104] reported that naphthalene radical ion in hexamethylphosphoric triamide cleaved methoxytrityl ether in high yield within 1 hr. Trityl derivatives have the useful property to act as markers on TLC upon reaction with dilute perchloric acid or with a ceric sulfate spray[59,106]; a yellow coloration appears due to formation of the triphenylmethyl cation. However, the removal of monomethoxytrityl group from nucleosides and nucleotides bearing benzoylated bases (purines) with 80% aqueous acetic acid is known to cause extensive depurination even at room temperature. Narang and his coworkers[105] have overcome this problem using a

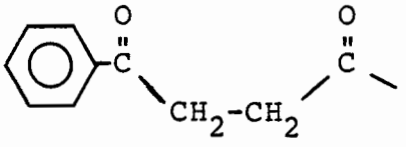
solution of 2% of benzesulfonic acid in a mixture of chloroform-methanol at 0°C for 20 min.

p-Bromophenacyloxytrityl group[106] was found to be very specific for the 5'-hydroxy functions. Its removal is accomplished by 40% acetic acid with zinc dust. Apparently this group offers very little advantage over the monomethoxytrityl group which appears to be stable enough and of reasonable lability. Therefore, the latter is the reagent of choice for 5'-protection. Moreover, the acidic removal of trityl ethers (AcOH 80%) doesn't affect significantly the stability of the phosphate protecting groups discussed above. Several other acid labile groups were introduced for the protection of 2'-, 3'- and 5'-hydroxy functions in the ribose series. 1-Ethoxyethyl[107] and 1-butoxyethyl acetals[108], tetrahydropyran-2-yl[58,109], 4-methoxytetrahydropyran-4-yl[110] and 2-methoxy-2-propyl[111] ketals have been commonly used.

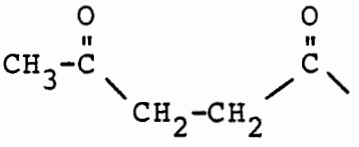
Esters have been frequently employed for the protection of 3'- and/or 5'-hydroxyls. Acylation is usually achieved by treatment of the nucleoside or nucleotide with an acyl anhydride or chloride in anhydrous pyridine[112-114]. Unfortunately these acylation reactions are not selective with respect to the 2'-, 3'- or 5'-position. Even free amino functions on the base moiety are often N-acylated under these conditions. A selective 5'-O-benzoylation was however

realized by the use of benzoic acid, diethyl azodicarboxylate and triphenylphosphine[115]. The acetyl group has been widely used by Khorana for the protection of the 3'-hydroxy function[16]. Several substituted acetyl groups were applied to oligonucleotide synthesis including trifluoroacetyl[116], phenoxyacetyl[117], p-chlorophenoxyacetyl[118], triphenylmethoxy-acetyl[58], 2-(p-tritylphenyl)sulfonyl ethyl[148] and methoxyacetyl moieties[118]. All of them were removed under mild basic conditions. It is noteworthy to mention that the p-nitro-phenyloxycarbonyl group[119] can be cleaved under extremely mild conditions such as treatment with imidazole in aqueous organic solvents.

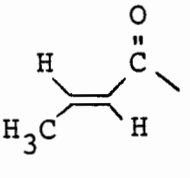
A group of hydroxy protecting groups which can be removed under neutral conditions has been investigated. The chloroacetyl group which lacks selectivity toward primary or secondary alcohol, was found to be cleaved by thiourea in ethanol[120]. Hydrazine hydrate is effective in the removal of benzoylpropionyl[143,144] (XLIV), levulinyl[145] (XLV) and crotonyl[146] (XLVI) moieties in pyridine/acetic acid buffer. Both acid and base sensitive linkages are unaffected under those conditions.



XLIV



XLV



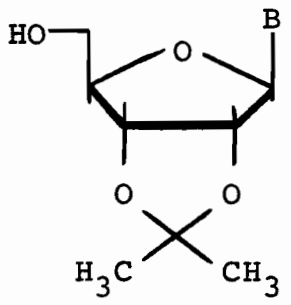
XLVI

2,2,2-tribomoethoxycarbonyl group[121] was found to be stable to acid, but can be removed by zinc-copper couple via a β-elimination.

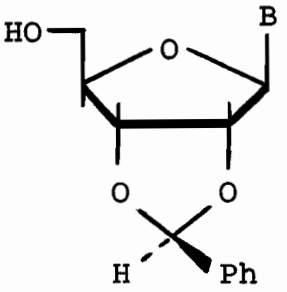
The tert-butyldimethylsilyl group was first employed by Corey for hydroxy protection in prostaglandin synthesis. Ogilvie et al.[123,124,126] extended the use of silyl groups in nucleoside and nucleotide chemistry. It appears that the silyl derivatives are relatively stable to base and hydrazine hydrate but are more sensitive to acid. The silylated nucleosides and/or nucleotides are deblocked by treatment with tetrabutylammonium fluoride in tetrahydrofuran. The deprotection is fast, clean and quantitative. It has been observed by Ogilvie's group[125] that the tert-butyldimethylsilyl, triisopropylsilyl and tetramethylene-tert-butylsilyl chlorides exhibit a high selectivity for the 5'-hydroxy function over the 3'-hydroxyl. The 5'-O-silyl derivatives were found to be more acid labile than the 3'-isomers. The silyl protecting group is gaining popularity in oligonucleotide synthesis via the diester approach. Recently the Khorana group[147] used the tert-butyldiphenylsilyl group as a masking group for the 3'-hydroxy function. The silyl ether combined with the 2-(p-tritylphenyl)sulfonyl ethyl group[148] greatly increased the lipophilicity of the diester and consequently the extraction of synthetic intermediates is greatly facilitated. Furthermore, the condensation products are readily separable

from starting material because of their uniquely strong retention on reverse-phase HPLC columns. It is also noteworthy to mention that the tert-butyldiphenylsilyl group has been successfully used in the protection of carbohydrate moieties[149].

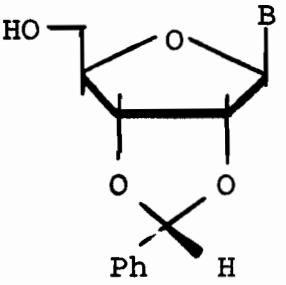
Very often in the ribose series, the simultaneous protection of the 2'- and 3'-hydroxyl functions may be desirable. 2',3'-O-isopropylidene ketal[127,128] (XLVII) and the diastereomeric benzylidene acetals[129] (XLVIII and XLIX) are commonly used for the selective protection of the cis-glycol system.



XLVII



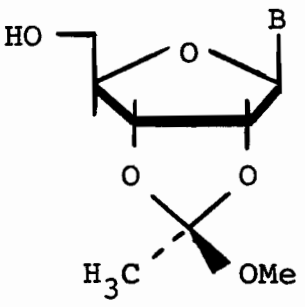
XLVIII



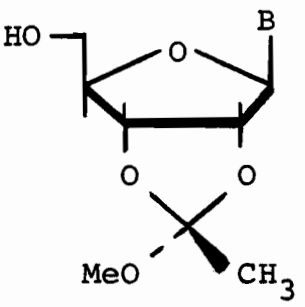
XLIX

Unfortunately, the removal of isopropylidene and benzylidene blocking groups under acidic conditions causes the 3'→2' migration of the phosphate backbone in certain ribonucleotides[130]. Therefore, p-dimethylamino- and 2,4-dimethoxybenzylidene

acetals which are more acid labile, have been recommended[131,132]. In the same vein, Reese[63] and others have used widely the diastereomeric methoxymethylidene acetals (L and LI) since they may be hydrolyzed under mild conditions involving sodium citrate buffer[109] (pH 5.6).



L



LI

The protection of the free amino-group of heterocyclic bases is required before the introduction of the hydroxyl protecting groups, in order to avoid producing N-substituted products when acylating reagents are used for the protection of hydroxyl functions. N-acetyl derivatives of nucleosides and nucleotides were extensively used by pioneering workers[133,134]. The instability of the N-acetyl group toward acidic conditions[135] as well as their limited solubility in pyridine restricted their use. Nevertheless, different acyl groups have been specifically used for the different bases, although the N-acyl derivatives make the glycosidic bond more susceptible to

hydrolytic cleavage especially in the deoxyribose series. Presently, anisoyl for cytosine[135], benzoyl for adenine[136] and isobutyryl[137] or 2-methylbutyryl for guanine seem to be the adequate protecting groups. The removal of these blocking groups is effected by concentrated ammonium hydroxide or with a 1:1 mixture of methanol and butylamine[103]. The dimethylaminomethylene group[138-142] was found to be adequate for the protection of amino groups in nucleoside and nucleotide synthesis. The protecting group was introduced in nearly quantitative yields from treatment of the appropriate nucleoside with dimethylformamide dimethyl acetal in dimethylformamide. The deblocking step occurs as well in acidic or basic solutions. The isobutyloxycarbonyl group[143] exhibits a great stability toward hydrazine where other amino protecting groups are cleaved. During oligonucleotide synthesis, if the benzoylpropionyl[144] group is used for masking the 3'-hydroxy function of cytidine for instance, the isobutyloxycarbonyl group will be the protecting group of choice for the N^4-amino group, because once protected, the benzoylpropionyl group could be removed by treatment with hydrazine without affecting the carbamate at the N^4-site. The latter is cleaved by concentrated ammonium hydroxide. This advantage is now outweighed by the use of the levulinyl group for the 3'-hydroxy function. Hydrazine cleaves that group so fast ($\sim$ 2 min) that the most commonly used N-acyl protecting groups are not significantly

affected by the reagent[145]. Narang et al.[166,167] reported dinucleotide synthesis via the phosphodiester approach involving the use of deoxyadenosine and deoxyguanosine without N-protecting groups and apparently, the yields obtained are the same as when the bases were protected. These observations are not however applicable to deoxycytidine which, without N-protection, gives unwanted side products. A thorough summary of protecting groups used in oligonucleotide synthesis has recently become available in an excellent review by Kössel and Seliger[174]. The internucleotide bond formation is so far the most important step in oligonucleotide synthesis. It is very important to obtain the highest yield of coupling in the minimum of time with the minimum amount of side products in order to save valuable starting material and to facilitate the purification of the product which is often a time consuming process. The coupling of a free phosphate function with a hydroxy function for the formation of a 3'-5' internucleotide bond is achieved with the aid of a condensing agent such as dicyclohexylcarbodiimide (DCC), mesitylene sulfonyl chloride (MS)[150] or 2,4,6-triisopropylsulfonyl chloride (TPS)[151]. Other activating agents such as carbonyl bis-(imidazole)[150], ethoxyacetylene[152], picryl chloride[153], mesitoyl chloride[154], trifluoroacetonitrite[155], cyclohexyl isocyanide[156] and N-ethyl-5-phenylisoxazolium fluoroborate[150,152] were found to be of limited interest. 2'-2'-Dipyridyl

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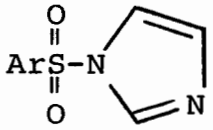
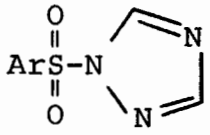
disulfide and triphenylphosphine were originally introduced for peptide synthesis[157], but were subsequently applied to the synthesis of mixed phosphate esters[158] and also to nucleotides[159,160]. Hata's group[161] introduced the combined use of triphenylphosphite and 2'-2'-dipyridyl diselenide as a coupling reagent in oligonucleotide synthesis. However, the general applicability of these condensing agents is still under investigation and consequently they are less used than MS and TPS. Nevertheless, MS and TPS present shortcomings which restrict their use. The production of hydrogen chloride during the condensation gives rise sometimes to unwanted side reactions. The sulfonation of hydroxyl functions is not completely eliminated even by using TPS. The most serious problem occurs during the purification step where the arylsulfonic acid is difficult to extract and causes messy separations, particularly for the triester approach. In order to circumvent these drawbacks, the employment of aryl-sulfonyl imidazolides LIIa-c as condensing agents[162] (Scheme X) was introduced although they were found to be generally slower than TPS for the formation of the phosphodiester linkages.

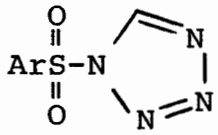
It can be expected that arylsulfonyl imidazolides will be even slower than TPS for the formation of triester linkages, because phosphodiester groups possess lower reactivity than monoesters. Nevertheless, sulfonation of hydroxyl functions was not detected, even with excess imidazolide and acid-

Scheme X

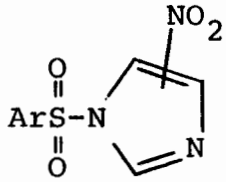




LIIIb-d



LIVb,c



LVc

LIIa, Ar = Tosyl

b, Ar = mesitylenesulphonyl

c, Ar = 2,4,6-triisopropylbenzenesulphonyl

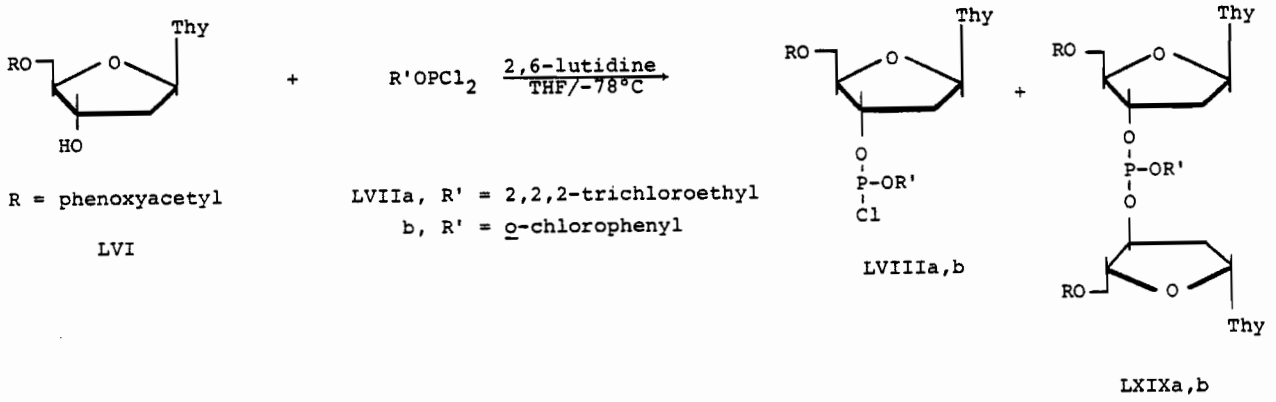
d, Ar = p-nitrobenzenesulphonyl

sensitive bonds in the starting materials were untouched. Shortly after the introduction of the imidazolide reagent, Narang's group[163-165] reported the use of arylsulphonyl-1,2,4-triazolides (LIIIb-d) as a novel condensing agent for polynucleotide synthesis. Compared with TPS, the rate of reaction using mesitylenesulfonyl triazolide (MST) or p-nitrobenzenesulphonyl triazolide (p-NBST) was rather slow but, the reaction mixture was apparently cleaner and the yield of the condensed product was appreciably higher especially for the sequences containing purine bases. The sulphonation side products were almost nonexistent, even after 5 days as compared with TPS reactions. In order to improve the reaction time in polynucleotide synthesis, Narang and his coworkers[166] reported the use of arylsulfonyltetrazoles (LIVb,c, Scheme X) as highly efficient condensing agents. These possess all the advantages of the arylsulfonylamides and proved to be faster than TPS for the formation of the phosphotriester linkage (30 min - 15 hr, depending upon the chain length). van Boom and Burgers[145] described the use of triisopropylbenzesulphonyl-4(5)-nitroimidazole LVc (TPSNI) as condensing agent in oligoribonucleotide synthesis. Apparently, TPSNI and Narang's tetrazolide have similar reactivity.

Recently, Letsinger and his collaborators[167,168] described the phosphite coupling procedure for the generation of nucleotidic linkages between deoxynucleosides. This procedure involves

the reaction of a suitably 5'-protected nucleoside (LVI) with an alkyl or aryl dichlorophosphite (LVIIa,b) (Scheme XI). The reaction occurred readily (∿ 5 min) in the presence of 2,6-lutidine at -78°C. The nucleoside protected at the 3'-position (LX) was then allowed to react under the same conditions (∿ 20 min) with the chlorophosphite LVIIIa,b. Oxidation of the resulting phosphite (LXIa,b) to the corresponding phosphate (LXIIIa,b) was effected by an aqueous solution of iodine (∿ 5-10 min). This procedure contrasts with those in which alkyl or aryl phosphorodichloridate have been used. These latter reactions were slow and low yields were obtained[56,62,63]. The advantages of the new procedure are the short time required for a complete reaction (∿ 35 min), the yield of the reaction which is comparable or even better than in any other procedures, and the facility of isolating the desired compound. Another advantage of the procedure is that adenosine derivatives can be used without protection of the free amino group[169]. When LVIIa is used as the phosphorylating agent, chains up to pentanucleotides using all four different bases were synthesized in good yield. The only disadvantage of the reaction is the production of 3'-3' (LXIX) and 5'-5' (LXII) isomers which are inevitable by using a symmetrical phosphorylating agent such as LVII. These minor side products can be easily separated from the 3'-5' isomer by chromatography on silica gel. The p-mono-

Scheme XI



R = phenoxyacetyl

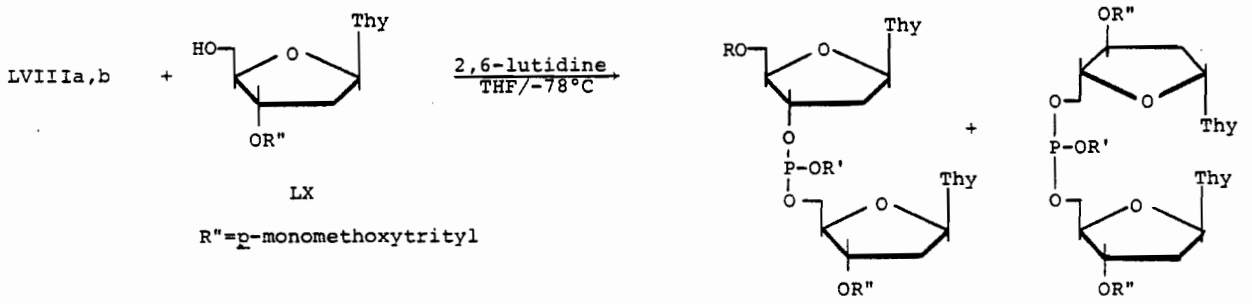
LVI

LVIIa, R' = 2,2,2-trichloroethyl

b, R' = o-chlorophenyl

LVIIIa,b

LXIXa,b

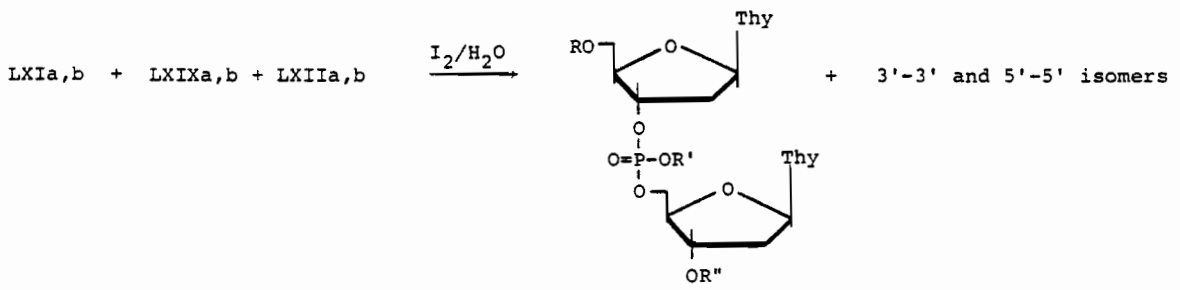


LX

R"=p-monomethoxytrityl

LXIa,b

LXIIa,b



LXIIIa,b

methoxytrityl group and the trichloroethyl phosphate protecting group are removed on the triester LXIIIa as well as on longer chains, by treatment with sodium-naphthalene in hexamethyl-phosphoric triamide (HMPA).

Although the phosphorylating agent LVIIb is not suitable for a trinucleotide synthesis[168] the more stable LVIIa may be advantageously used for this purpose. van Tamelen[98] and Ogilvie[170,171] have already applied the phosphite triester approach to oligoribonucleotide synthesis.

The "modified triester approach" and especially the phosphite coupling procedure seem to be the methods of choice for the large scale production of polynucleotides in the minimum of time. The production of phosphotriesters is at present satisfactory but the availability of fully deprotected polynucleotides is even more desirable. Thus, the deprotection procedure becomes as valuable as the phosphotriester synthesis itself. The ideal deblocking method for oligonucleotide triesters is the one which will remove all the protecting groups in one step under the mildest conditions. The purpose of our studies will therefore be involved in the finding of an agent which will be potentially able to effect a clean and fast deprotection on polynucleotide triesters. As was remarked in this brief review, fluoride ion nicely cleaves the silyl ether derivatives. With this basis, we will study the behavior of the silyl group as a

hydroxy function protecting group for the conditions used in oligonucleotide synthesis. The fluoride ion promoted desilyation will be examined with respect to different phosphate protecting groups. From these observations, it will be hopefully possible to judge the potential of fluoride ion and to envisage certain modifications on the nucleotide synthesis which will permit a better subsequent deprotection by fluorides. As a second goal for our studies, we wish to introduce fluoride ion as a useful reagent for the synthesis of oligonucleotide analogs which are often used for testing the validity of certain biologically important mechanisms.

RESULTS AND DISCUSSION

The tert-Butyldimethylsilyl Group as a Protecting Group in Deoxynucleotide Synthesis

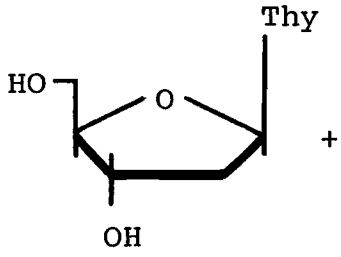
1. Silylation of Deoxynucleosides

The strategy to be used in our studies will involve the protection of the 5'-hydroxyl function of thymidine and deoxycytidine (dC) with a p-monomethoxytrityl group (mmt) or with a p-dimethoxytrityl group (dmt). The phosphorylated derivatives will be reacted with the 3'-tert-butyldimethylsilyl (TBDMS) derivatives of the parent nucleosides to give dinucleotides.

For this purpose, thymidine (LXIV) was tritylated and isolated according to Khorana's procedure[172]. The silylation of 5'-O-dimethoxytritylthymidine (LXVa) with tert-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in N,N-dimethylformamide (DMF) was achieved according to Ogilvie's method[173]. The fully protected nucleoside (LXVIa) was then treated with an aqueous solution of 80% (v/v) acetic acid (AcOH) (Scheme XII).

The complete removal of the dmt group was nearly complete after 1.5-2 hr of stirring at room temperature. After evaporation of the acetic acid in excess under vacuo, the desired 3'-O-tert-butyldimethylsilyl thymidine (LXVII)

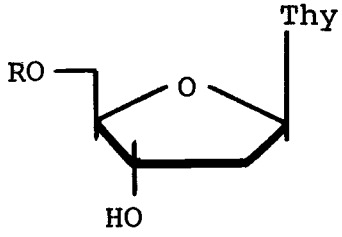
Scheme XII



LXIV

+ dmtCl or mmtCl

Pyridine, R.T.

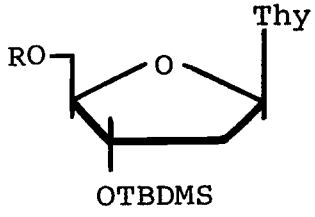


LXVa, R = dmt

b, R = mmt

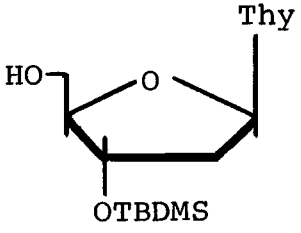
LVa,b + TBDMSCl

Imidazole, DMF



LXVIa,b

AcOH 80%, R.T.



LXVII

was obtained from a short column chromatography in yields varying from 80-90% based on LXV. The yields are slightly lower (65-75%) when LXVb is used as starting material because it takes longer to remove the p-monomethoxytrityl group which allows to a greater extent the removal of the 3'-O-TBDMS group.

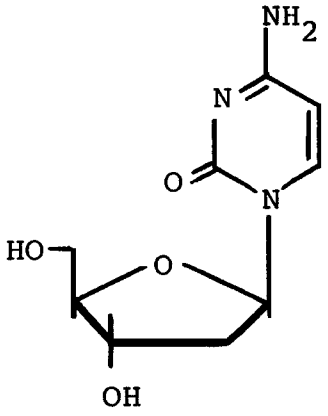
Due to the phosphorylation and tritylation requirements, the free amino group of deoxycytidine has to be protected. The N-benzoyl group is known to be adequate for our purposes. Deoxycytidine (LXVIII) was fully benzoylated by treatment with benzoyl chloride (BzCl) in pyridine according to Khorana's procedure[172]. The fully protected deoxycytidine (LXIX) was then selectively saponified to the desired N-benzoyldeoxycytidine (LXX) according to the latter procedure.

LXX was tritylated, silylated and then detritylated under similar conditions as for the deoxythymidine case (Scheme XIII). Similar yields of N-benzoyl-3'-O-tert-butyl-dimethylsilyl deoxycytidine (LXXI) were obtained.

2. Procedure for the Generation of Internucleotide Links by using β-Cyanoethylphosphate (βCEP) (Scheme XIV)

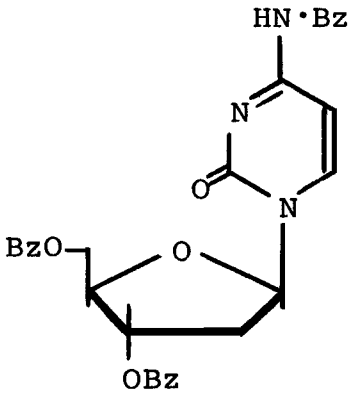
The methodology for this kind of phosphorylation involves two steps[40]. The first one consists of the phosphorylation coupling of the 5'-O-monomethoxytrityl nucleoside with the

Scheme XIII



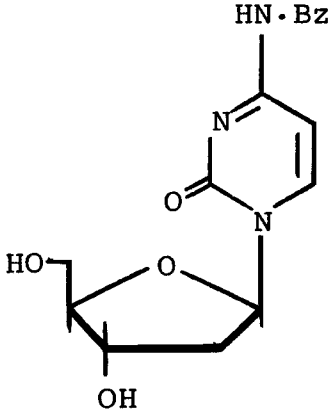
LXVIII

BzCl
Pyridine



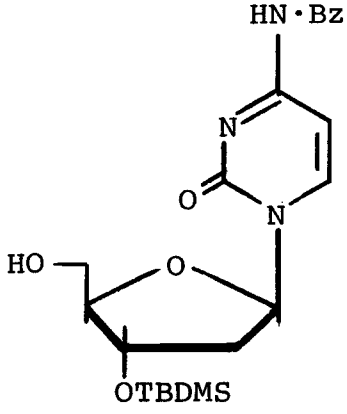
LXIX

NaOH
5°C
5-10 min



LXX

1. dmtCl/Pyr
2. TBDMSCl
   Imidazole/DMF
3. AcOH 80%



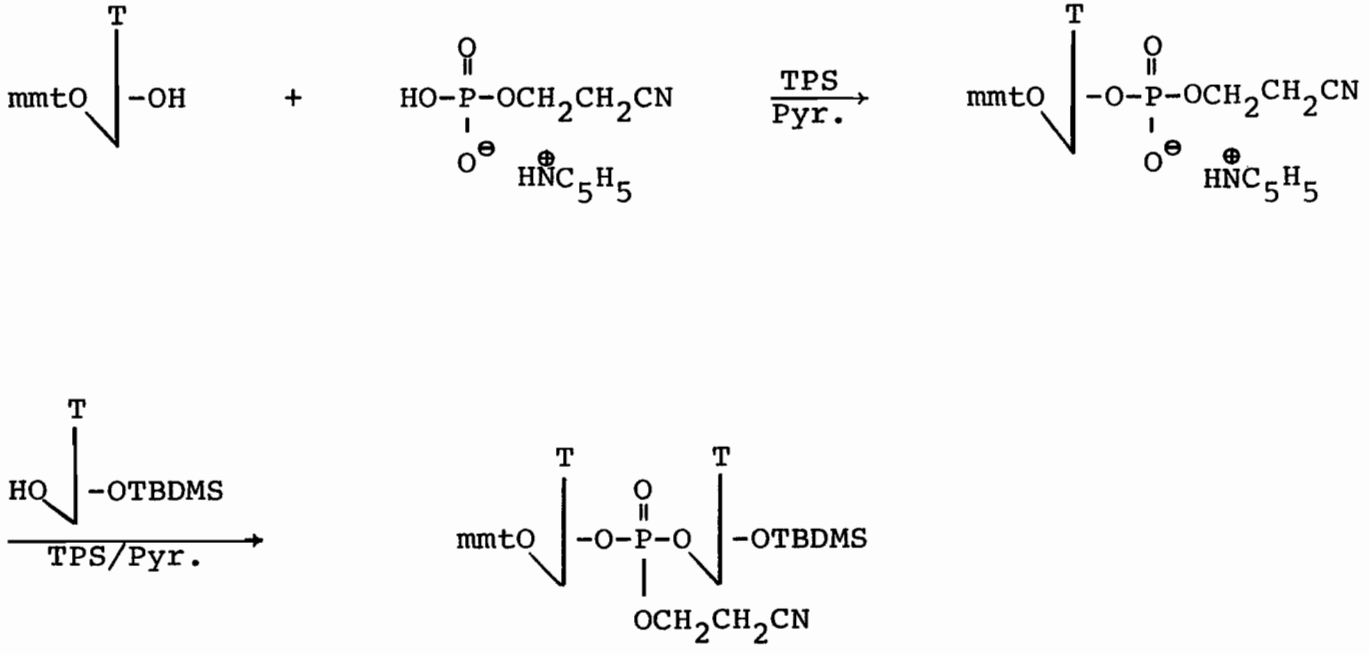
LXXI

pyridinium salt of β-cyanoethylphosphate in the presence of triisopropylbenzenesulphonyl chloride (TPS). The resulting diester is then coupled in the last step with the 3'-O-TBDMS nucleoside via a TPS assisted condensation. The purification of the desired triester is accomplished on silica gel TLC plates. The presence of sulphonic acid complicates the purification and often a second purification is required.

Habitually the phosphotriester is precipitated as a white powder by addition of hexanes. Yields vary between 40-60%. Scheme XIV illustrates the preparation of the β-cyanoethyl-phosphate derivative of 5'-O-p-monomethoxytrityl thymidilyl-(3'→5')-3'-O-tert-butyldimethylsilyl thymidine (mmtTp(CE)TSi).

According to this procedure, mmtTp(CE)TSi, mmtTp(CE)dC^{Bz}Si and mmtdC^{Bz}p(CE)TSi were prepared in 52%, 40% and 41% yield respectively, based on their limiting starting materials. No apparent desilylation was observed after the phosphorylation step and after purification from silica gel. Extension of the nucleotide chain will therefore require the selective removal of a protecting group from one of the two terminal hydroxy functions. It turns out that the p-monomethoxytrityl group can be removed selectively by treatment of the fully protected dinucleotide with 80% aqueous acetic acid at ambient temperature for 2.5-3 hr. Tp(CE)TSi, Tp(CE)dC^{Bz}Si and dC^{Bz}p(CE)TSi were obtained in yields varying from 85-95%, suggesting that the 3'-O-TBDMS group is quite stable under these conditions.

Scheme XIV



Use of heat for increasing the rate of detritylation is not recommended since it enhances desilylation and promotes the N-glycoside bond cleavage on N-protected deoxycytidine and/or similarly protected purine bases.

The stepwise synthesis of trinucleotide is achieved by the two step condensation of the suitably protected nucleoside with the 5'-unblocked dinucleotide. The procedure and the reaction conditions are similar to those used for the dinucleotide synthesis. mmtTp(CE)Tp(CE)TSi and mmtdC^{Bz}p(CE)-Tp(CE)TSi were isolated in 50% and 42% yield respectively based on the 3'-O-TBDMS dinucleotide derivatives. The latter was deprotected by successive treatment with tetrabutyl ammonium fluoride (TBAF) in THF, concentrated ammonium hydroxide (NH_4OH) in pyridine and finally by 80% aqueous acetic acid. The deprotected nucleotide was then passed through a column filled with an ion-exchange resin (DOWEX50W-X8 $Na^{\oplus}$ ionic form) in order to transform the excess TBAF into the less lypophilic sodium fluoride (NaF) to permit an easier subsequent purification of the trinucleotide on preparative paper chromatography (solvent A). After isolation from papers, the pure dCpTpT was subjected to enzyme degradations by both bovine spleen and snake venom phosphodiesterases. dCpTpT was fully degraded by both enzymes in proper ratios (see Experimental section).

As we have discussed in the Introduction, the stepwise

approach is valuable but is still a time-consuming process. Since we showed that the mmt group can be removed selectively from a dinucleotide, we may envisage the selective removal of the TBDMS group from another dinucleotide and if the process proved successful, the blockwise polynucleotide synthesis would then be accessible.

When the fully protected dinucleotide mmtTp(CE)TSi was treated with ten molar equivalents of TBAF in THF, the TBDMS group was indeed removed, but we observed also the removal of the cyanoethyl phosphate protecting group. Fluoride ion is a strong nucleophile and also behaves as a strong base in aprotic solvents[175]. We found as well as other workers[176-179] did before that glacial acetic acid strongly solvates fluoride ion reducing consequently its nucleophilicity and more drastically its basicity. So, when the dinucleotide mmtTp(CE)-TSi was treated with a solution of ten molar equivalents of TBAF and four hundred molar equivalents of glacial acetic acid in THF, mmtTp(CE)T was formed in ca. 80-85% yield, ca. 5% of starting material was left over after 24-30 hr and ca. 10% of cyanoethylphosphate protecting group was cleaved during this period of time. Unfortunately, the selectivity in the removal of the TBDMS group decreases with increasing chain length. When the trinucleotide mmtdC^{Bz}p(CE)Tp(CE)TSi was reacted with a solution of ten molar equivalents of TBAF and four hundred molar equivalents of phenylacetic acid (PAA)

in THF, the best yield of mmtdC^{Bz}p(CE)Tp(CE)T (ca. 55%) was observed after 37 hr.; ca. 5% of starting material was left over and ca. 40% of cyanoethyl group cleavage was noticed. In light of the latter results, the selective removal of TBDMS group occurred satisfactorily on the dinucleotide only when CE was used as protecting group. Nevertheless, the TBDMS group is compatible with the mmt group and the cyanoethyl-phosphate protecting group for a stepwise or a blockwise oligonucleotide synthesis. In order to estimate the scope of the applicability of the TBDMS group, we wished to check its compatibility with other phosphate protecting groups such as the well-known phenyl and trichloroethyl groups.

3. General Procedures in the Use of Phenyl and Trichloroethyl Moieties as Phosphate Masking Groups in Polynucleotide Synthesis

The technique used for our forthcoming nucleotide synthesis will be closely related to Reese's technology[180]. A properly 5'-protected nucleoside was phosphorylated by diphenylchlorophosphate or by bis(2,2,2-trichloroethyl) phosphorochloridate in pyridine. This kind of phosphorylation can be carried out on very large scale without any complications. The resulting triesters were purified easily from dry silica gel chromatography and were isolated in

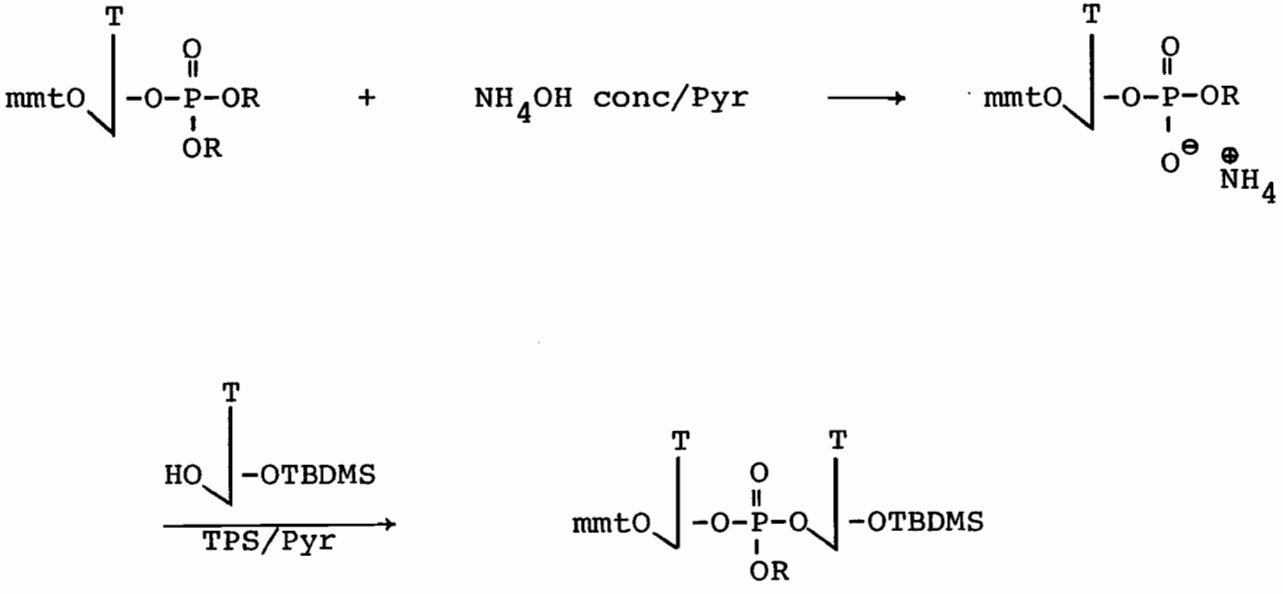
nearly quantitative yields. The triesters were converted to diesters by treatment with concentrated ammonium hydroxide in pyridine. For instance, the bis-phenyl phosphate derivative of 5'-O-p-methoxytrityl thymidine (mmtTp$^{\phi}_{\phi}$) was converted into its corresponding diester in 96.4% yield after 8 hr at room temperature. 3.6% of sugar-phosphate bond breaking occurred during the reaction as evidenced by the presence of 5'-O-p-monomethoxytritylthymidine (mmtT) on TLC. These results agree with those reported by Reese[181]. On the other hand, the bis(2,2,2-trichloroethyl) phosphate analog (mmtTp$^{TCE}_{TCE}$) was converted into its diester in 81.1% yield after 1.5 hr at room temperature. 18.9% of mmtT was formed via sugar-phosphate bond breaking. These yields were recorded spectrophotometrically after detritylation of the reaction mixture with 80% aqueous acetic acid. Test reactions indicated that the acidic removal of mmt group did not promote phosphate ester hydrolysis on the fully protected triesters and diesters respectively. Purification of the diesters was easily performed on short silica gel column chromatography. The diesters were then dried with the appropriate 3'-O-TBDMS nucleoside (0.5 molar equivalent) by usual coevaporation with dry pyridine. TPS (2 molar equivalents) was added, followed by the minimum amount of dry pyridine. The solution was stirred for 36 hr at ambient temperature. Standard hydrolysis, work-up procedure and purification on

thick layer silica gel plates were applied (see experimental section). Scheme XV exemplifies well the procedure used.

mmtTp(ϕ)TSi, mmtTp(ϕ)dC^{Bz}Si and mmtTp(TCE)TSi were synthesized in 73%, 46% and 65% yield based on their 3'-O-TBDMS nucleoside precursors. As demonstrated previously, the selective removal of the mmt group occurs in high yields (90-95%). From the stepwise approach, mmtTp(ϕ)Tp(ϕ)TSi, mmtTp(ϕ)Tp(ϕ)Tp(ϕ)TSi, mmtdC^{Bz}p(ϕ)Tp(ϕ)dC^{Bz}Si, mmtTp(ϕ)dC^{Bz}p(ϕ)Tp(ϕ)dC^{Bz}Si and mmtTp(TCE)-Tp(TCE)TSi were prepared in 69%, 44%, 24%, 37% and 72% yield respectively based on their 3'-O-TBDMS nucleotide precursors. Here again, the compatibility of the TBDMS group with mmt group, phenyl and trichloroethyl phosphate protecting groups is perfect for a stepwise polynucleotide synthesis.

It is significant that when mmtTp(ϕ)TSi, mmtTp(TCE)TSi or mmtTp(ϕ)dC^{Bz}Si was treated with ten molar equivalents of TBAF in dry THF, both silyl and phosphate protecting groups were removed within 60 min. Therefore, fluoride ion may be used for the removal of phosphate protecting groups and marks a major improvement in the removal of the trichloroethyl group where yields in the zinc catalyzed cleavage are often very low[60]. Interestingly, when the latter reactions were carried out in the presence of 200 molar equivalents of phenylacetic acid (PAA) at room temperature for 20-24 hr, mmtTp(TCE)T, mmtTp(ϕ)T, and mmt-Tp(ϕ)dC^{Bz} were isolated from thick layer silica gel plates in 90%, 89% and 95% yield respectively. Alternatively, mmtTp(TCE)TSi was treated with

Scheme XV



R = phenyl, 2,2,2-trichloroethyl.

ten molar equivalents of TBAF in the presence of 400 molar equivalents of glacial acetic acid in dry THF. The reaction mixture was stirred at room temperature for a day and was then left without stirring for five more days. The mixture was then worked up and treated with 80% acetic acid. Two bands were separated on preparative paper chromatography and their yields were recorded spectrophotometrically. 87.6% of Tp(TCE)T and 12.4% of TpT were obtained. In other words, the desilylation was complete and only 12% of the trichloroethyl protecting group was removed under these conditions even after 6 days. From these data, one can conclude that phenyl or trichloroethyl phosphate protecting groups are the groups of choice for both stepwise and blockwise synthesis of oligonucleotides when mmt and TBDMS are used as hydroxy function protecting groups. Definitely, the advantages of using the TBDMS group in polynucleotide synthesis lie in its facility of introduction and its stability to various phosphorylation conditions including those related with the new phosphite coupling procedure[170,171]. The selective removal of TBDMS group by solvated fluorides in the presence of a variety of phosphate protecting groups makes its use very attractive for the preparation of oligodeoxynucleotides where the blockwise synthesis is recommended. The next section will be concerned with the removal of phosphate protecting groups by fluorides. Our attention will be focussed on the amount of internucleotidic

bond breaking obtained with respect to standard deprotection procedures.

Studies on the Deprotection of Mono- and Dinucleotide Triesters by Fluorides in Aprotic Solvents

1. Deprotection of Mononucleotides

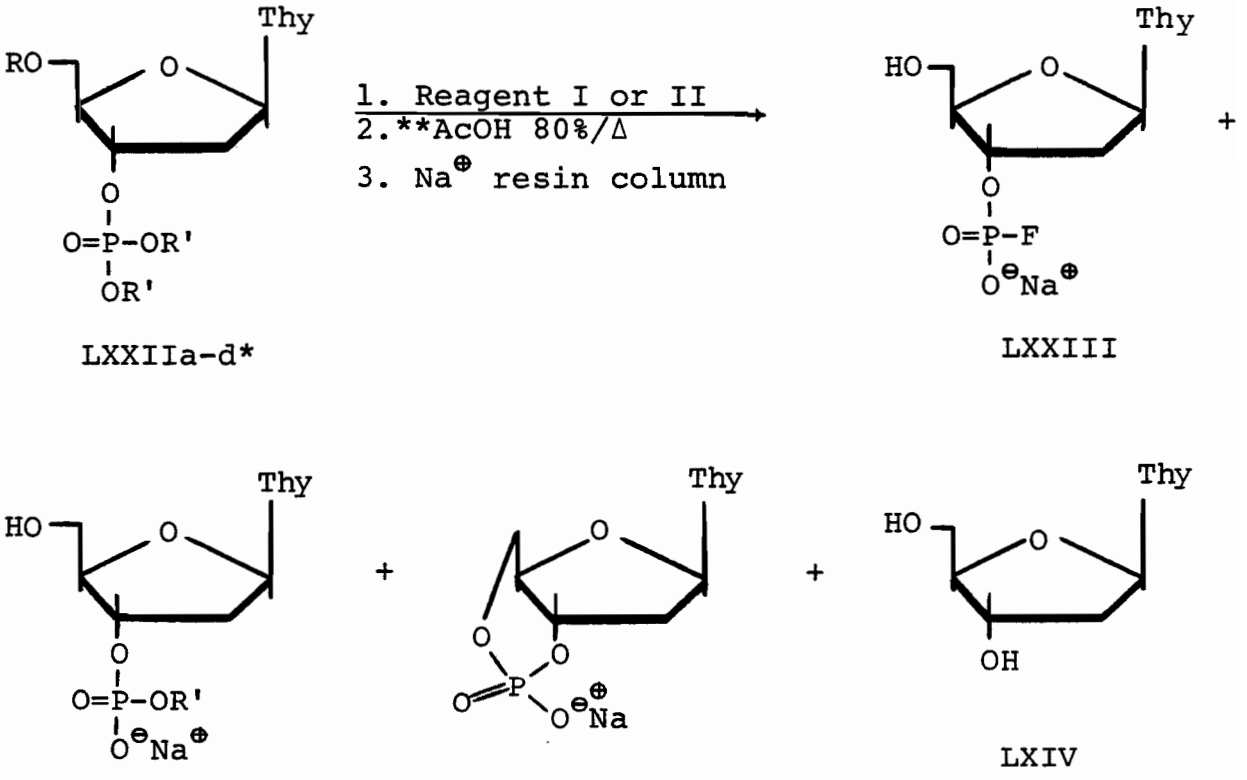
It was shown in the last section that fluoride ion in dry THF (Reagent I) was able to cleave not only the silyl protecting group on dinucleotide triesters, but also the phenyl, trichloroethyl and cyanoethyl phosphate protecting groups. At least for phenyl and trichloroethyl phosphotriesters, fluoride ion attacks the phosphate moiety displacing the protecting group, since phenol and 2,2,2-trichloroethanol are detected by gas chromatography analysis[182].

These preliminary observations prompted us to similarly deprotect symmetrical phosphotriesters such as LXXIIa and LXXIIb (Scheme XVI). During our investigation, Narang and coworkers[183] published a paper relating the use of fluoride ion in a solution of tetrahydrofuran, pyridine and water (8:1:1) for the deprotection of polynucleotide triesters. We decided to use Narang's reagent (Reagent II) as well and then compare the results with those obtained with Reagent I. For this purpose, all the deprotection reactions were worked-up by passing them through a column filled with an ion-exchange resin ($Na^{\oplus}$ ionic form, DOWEX 50W-X8) in order to get rid of excess TBAF*, thus

*According to the procedure of A.L. Schifman of our laboratory.

facilitating the chromatography on preparative papers. The yields of the bands are recorded spectrophotometrically. The results for the deprotection of both mono- and dinucleotides are given in Tables I and VIIIa.

Scheme XVI



LXXIIa-d*

LXXIII

LXXIVa,b*

LXXV

LXIV

*a, R=mmt, R'=ϕ; b, R=mmt, R'=TCE; c, R=H, R'=ϕ; d, R=H, R'=TCE.

**Required only for LXXIIa and LXXIIb.

When the bis-phenyl ester derivative of 5'-O-p-monomethoxytrityl thymidine-3'-phosphate (mmtTp^{ϕ}_{ϕ}, LXXIIa) was treated with six molar equivalents of TBAF in dry tetrahydrofuran (Reagent I) for 30 min, the fluorophosphate LXXIII (Tp^{F}_{OH}) was isolated in 84.5% yield along with 6.0% of the diester LXXIVa (Tp^{ϕ}_{OH}) after acidic work-up. Paper chromatographic, electrophoretic and spectral properties of LXXIII are identical to those of an authentic sample synthesized according to a procedure in the literature[184]. (LXXIVa,b were prepared from the alkaline hydrolysis of LXXIIa,b followed by treatment with 80% aqueous acetic acid). Sugar-phosphate bond breaking was also observed as evidenced by the presence of thymidine (LXIV, 9.5%). If Reagent II was used instead, LXXIII was formed in 74.4% after 24 hr along with 6.9% of LXXIVa and 18.6% of thymidine. It is interesting to note that for this case, Reagent II gives the most sugar-phosphate bond breaking.

Using LXXIIb (mmtTp^{TCE}_{TCE}) as starting material with Reagent I, only 34.3% of LXXIII was observed after 30 min. However, 56.8% of LXXIVb (Tp^{TCE}_{OH}) was formed along with 8.9% of thymidine. This suggests that the trichloroethyl phosphate protecting group is more resistant to fluoride attack than is the phenyl analog. This trend is exemplified by the reaction of LXXIIb with Reagent II. After 24 hr, LXXIVb was formed in 73.2% along with 15.8% LXXIII. 10.9% of thymidine was also observed.

The 5'-unblocked triesters LXXIIc (Tp^{ϕ}_{ϕ}) and LXXIId (Tp^{TCE}_{TCE})

were also treated with Reagent I. After 30 min, LXXIII was formed in 77.8% yield if LXXIIc was used as starting material. No traces of LXXIVa were detected. However, the formation of a new compound (10.2%) was observed. The latter was identified as the cyclic phosphate LXXV (cTMP). Paper chromatographic and electrophoretic properties of LXXV were identical with those of an authentic sample (Sigma Co.). Presumably, LXXV is obtained through the formation of a hydrogen bond between fluoride ion and the 5'-hydroxy group causing the 5'-oxygen to become more nucleophilic. The 5'-oxygen then attacks the phosphate centre affording eventually the cyclic phosphate LXXV. The same trend is observed with LXXIId as starting material except for the formation of LXXIVb which occurred in 45.9% yield. The reaction of LXXIIc with Reagent II gave no detectable amount of cTMP, but gave 81.3% of Tp^{F}_{OH} along with 16.2% of sugar-phosphate bond breaking. The presence of water in this reaction mixture inhibits the formation of the cyclic phosphate LXXV. Using LXXIId as starting material, the formation of cTMP was nonexistent, but Tp^{TCE}_{OH} was formed in 70.4% yield contrasting with the 2.5% of Tp^{ϕ}_{OH} obtained when LXXIIc was used as starting material.

In light of the above results, it is clear that fluoride ion (Reagent I and II) is not suitable for the generation of adequate amounts of diesters such as the 5'-O-p-monomethoxy-trityl derivatives of LXXIVa and LXXIVb which might be useful

in polynucleotide syntheses. However, the fluoride assisted deprotections of LXXIIa and LXXIIc with Reagent I and Reagent II respectively give a simple and fast (Reagent I) synthesis of Tp^{F}_{OH} which is usually prepared from the expensive thymidine-3'-phosphate (Tp) and 1-fluoro-2,4-dinitrobenzene[184]. It is noteworthy to mention that the 5'-O-p-monomethoxytrityl derivative of LXXIII has been used in the synthesis of polynucleotide diesters[185-186]. The use of $mmtTp^{F}_{OH}$ in the synthesis of a "charged trinucleotide" and its usefulness in the elucidation of the mechanism of nucleotide triesters deprotection will be discussed later in this section.

2. Deprotection of Dinucleotides

The fully protected dinucleotides LXXVIa-c and the 5'-unblocked dinucleotides LXXVId-f (Scheme XVII) were deprotected with both Reagent I and Reagent II. With Reagent I the deprotection reactions were for 1 hr duration and 24 hr with Reagent II. The deprotection reactions were then processed as above. The results are reported in Table I.

One of the major concerns of any procedure for the deprotection of nucleotides is the possibility of cleavage of internucleotide bonds. As shown in Scheme XVII any cleavage of the 3'-5' internucleotide bond must produce thymidine (LXIV) along with other fragments such as Tp^{F}_{OH} (LXXIII) and $_{HO}{}^{F}pT$

Table I. Deprotection of Mono- and Dinucleotide Triesters by Fluoride Ions in Aprotic Solvents

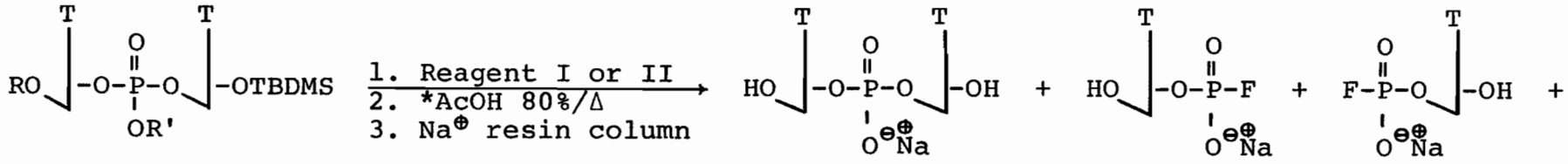
Starting Material	Reagent	Time	O.D. Units (Product*, % yield)			
mmtTp$^{\phi}_{\phi}$ (LXXIIa)	I	30 min	82.8 (Tp$^{F}_{OH}$, 84.5)	5.89 (Tp$^{\phi}_{OH}$, 6.0)	9.27 (T , 9.5)	
mmtTp$^{\phi}_{\phi}$ (LXXIIa)	II	24 hr	68.2 (Tp$^{F}_{OH}$, 74.4)	6.37 (Tp$^{\phi}_{OH}$, 6.9)	17.1 (T , 18.6)	
Tp$^{\phi}_{\phi}$ (LXXIIc)	I	30 min	64.1 (Tp$^{F}_{OH}$, 77.8)	8.40 (cTMP, 10.2)	9.85 (T , 11.9)	
Tp$^{\phi}_{\phi}$ (LXXIIc)	II	24 hr	86.0 (Tp$^{F}_{OH}$, 81.3)	2.61 (Tp$^{\phi}_{OH}$, 2.5)	17.1 (T , 16.2)	
mmtTp$^{TCE}_{TCE}$ (LXXIIb)	I	30 min	63.7 (Tp$^{TCE}_{OH}$, 56.8)	38.4 (Tp$^{F}_{OH}$, 34.3)	9.96 (T , 8.9)	
mmtTp$^{TCE}_{TCE}$ (LXXIIb)	II	24 hr	70.2 (Tp$^{TCE}_{OH}$, 73.2)	15.2 (Tp$^{F}_{OH}$, 15.8)	10.5 (T , 10.9)	
Tp$^{TCE}_{TCE}$ (LXXIId)	I	30 min	45.2 (Tp$^{TCE}_{OH}$, 45.9)	39.3 (Tp$^{F}_{OH}$, 39.9)	5.25 (cTMP, 5.3)	8.7 (T , 8.8)
Tp$^{TCE}_{TCE}$ (LXXIId)	II	24 hr	63.7 (Tp$^{TCE}_{OH}$, 70.4)	17.7 (Tp$^{F}_{OH}$, 19.6)	9.1 (T , 10.0)	
mmtTp(ϕ)TSi (LXXVIa)	I	60 min	71.4 (TpT, 86.9)	5.36 (T , 6.5)		
mmtTp(ϕ)TSi (LXXVIa)	II	24 hr	84.9 (TpT, 92.2)	3.60 (T , 3.9)		
mmtTp(ϕ)TSi (LXXVIa)	**	24 hr	81.3 (TpT, 91.9)	3.56 (T , 4.0)		
Tp(ϕ)TSi (LXXVId)	I	60 min	43.9 (TpT, 63.2)	16.5 (X†, 23.8)	4.51 (T , 6.5)	
Tp(ϕ)TSi (LXXVId)	II	24 hr	60.1 (TpT, 93.1)	2.21 (T , 3.4)		
mmtTp(TCE)TSi (LXXVIb)	I	60 min	77.7 (TpT, 87.9)	5.37 (T , 6.1)		
mmtTp(TCE)TSi (LXXVIb)	II	24 hr	77.2 (TpT, 78.1)	3.1 (T, 3.2)	15.4 (Tp(TCE)T, 15.6)	
Tp(TCE)TSi (LXXVIe)	I	60 min	65.2 (TpT, 74.8)	9.47 (X†, 10.8)	6.24 (T , 7.2)	
Tp(TCE)TSi (LXXVIe)	II	24 hr	48.1 (TpT, 55.2)	33.9 (Tp(TCE)T, 38.8)	2.59 (T , 3.0)	
mmtTp(CE)TSi (LXXVIc)	I	60 min	64.3 (TpT, 85.1)	11.3 (Tp$^{CE}_{OH}$ or $^{CE}_{HO}$pT + T , 14.9)		
mmtTp(CE)TSi (LXXVIc)	II	24 hr	quantitative yield of TpT			
Tp(CE)TSi (LXXVIf)	I	60 min	49.7 (TpT, 78.9)	13.3 (Tp$^{CE}_{OH}$ or $^{CE}_{HO}$pT + T , 21.1)		
Tp(CE)TSi (LXXVIf)	II	24 hr	92.2 (TpT, 95.3)	2.28 (T , 2.4)		
mmtU^{Si}p(TCE)U$^{Si}_{Si}$ (LXXVIII)	I	30 min	54.1 (mmtUpU, 85.7)	4.50 (U, 7.1)		
mmtU^{Si}p(TCE)U$^{Si}_{Si}$ (LXXVIII)	II	24 hr	74.9 (mmtUpU, 76.5)	4.25 (U, 4.3)	14.5 (mmtUp(TCE)U, 14.8)	

*After detritylation when required.

**TBAF in THF containing 5% (v/v) of added water.

†X has similar chromatographic, electrophoretic and spectral properties as for TpT (see Table VIIIa).

Scheme XVII



LXXVI a, R=mmt, R'=ϕ
b, R=mmt, R'=TCE
c, R=mmt, R'=CE
d, R=H, R'=ϕ
e, R=H, R'=TCE
f, R=H, R'=CE

LXXVII + LXXIII + LXXIIIi

(**) + (**) + LXIV

---

* Required only for LXXVIa-c.

** Obtained only from LXXVIc or LXXVIf.

(LXXIIIi). It is only necessary to determine the spectrophotometric yield of thymidine and multiply by two to determine the percentage of chain cleavage*.

The reaction of LXXVIa with Reagent I gave the desired TpT (LXXVII) in 86.9% along with 13% of chain cleavage. Under similar conditions, Reagent II produced 92.2% of TpT and 7.8% of internucleotide bond breaking. Using LXXVIb as starting material with Reagent I, similar results as for the LXXVIa case were observed although slightly less (12.1%) chain cleavage was detected. However, Reagent II is slower to react with LXXVIb. After 24 hr, 78.1% of LXXVII was produced along with 6.4% of chain cleavage. 15.6% of Tp(TCE)T remained unreacted.

The reaction of LXXVIc with Reagent I gave 85.1% of TpT and 14.9% of a mixture of thymidine, Tp^{CE}_{OH} and $^{CE}_{HO}pT$. These results are surprising since the fluoride assisted removal of the cyanoethyl phosphate protecting group occurs via a β-elimination mechanism with concomitant formation of acrylonitrile (Eq. 1).

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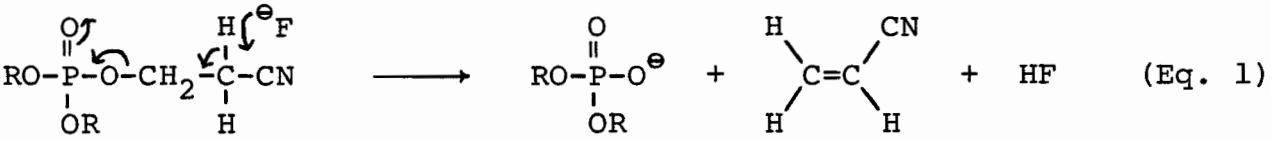
*The u.v. absorbance of one thymidine unit is approximately half the absorbance of one TpT unit. Yields of products in these reactions are calculated from the number of O.D. units of each product obtained. The number of O.D. units of thymidine always is equal to the number of O.D. units of Tp^{F} and $^{F}_{HO}pT$ or Tp^{CE}_{OH} and $^{CE}_{HO}pT$ depending on which case is examined.



(Eq. 1)

On the other hand LXXVIc and Reagent II reacted together to produce a quantitative yield of TpT without any detectable amount of thymidine or other side products. In the light of these results with all the protecting groups it is obvious that the reaction of LXXVIa-c with Reagent I gave more internucleotidic bond breaking than with Reagent II. This point was raised recently by van Boom and Burgers[187].

The results obtained here contrast with those reported in our preliminary communication[182] where no chain cleavage was indicated. However, it is possible to explain the discrepancy in the results. In our preliminary communication, the preparation of TBAF was not as carefully carried out to eliminate moisture. This point is very important because we carried out a reaction involving LXXVIa and TBAF in dry THF containing 5% (v/v) of added water. From this reaction we obtained 91.9% of LXXVII and 8.0% of chain cleavage. The last results are virtually identical to those obtained by the reaction of LXXVIa with Reagent II which contains 10% water.

As a result, it is likely that in our initial experiments there was as much as 8-10% chain cleavage. The cleavage products were not detected since the reaction mixture was directly applied to preparative Whatman 3MM paper which was

developed in Solvent A. It was subsequently discovered that the TBAF present in these mixtures causes thymidine, TpT and other degradation products to have the same mobility. Only after TBAF was eliminated by the ion-exchange procedure did we notice the small amounts of thymidine and other degradation products.

The reaction of LXXVId with Reagent I produced 63.3% of TpT, 23.7% of a new compound and 13% of internucleotide bond breaking. The production of a new material (10.9%) also occurred when LXXVIe was used as starting material. With LXXVIf and Reagent I, in addition to the deprotected dinucleotide, the formation of thymidine and Tp^{CE}_{OH} or $^{CE}_{HO}pT$ was observed in 21.1% yield. The generation of the latter is greatly reduced by the use of Reagent II. For instance, TpT was formed in 93.1% along with 6.9% of chain cleavage when LXXVId was employed as starting material. As usual, Reagent II is slow to react with trichloroethylphosphotriesters. Indeed, with LXXVIe, only 55.2% of LXXVII was produced along with 5.9% of chain cleavage; 38.8% of Tp(TCE)T was left over. The reduced nucleophilicity of fluoride ions in Reagent II improved considerably the deprotection of LXXVIf from which 95.3% of TpT was produced with 4.7% of internucleotide bond breaking.

With 5'-unblocked dinucleotide triesters (LXXVId-f) Reagent II gave better results and cleaner reaction products than those obtained with Reagent I. With 5'-blocked dinucleotide

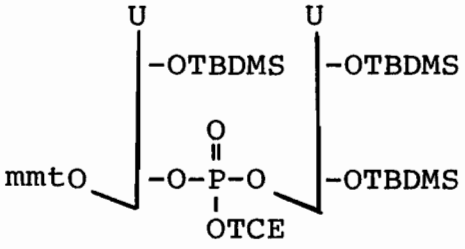
triesters (LXXVIa-c), Reagent II is slightly superior to Reagent I for the deprotection in terms of internucleotide bond breaking regardless of the results obtained with LXXVIc, which come from a different deprotection mechanism. In addition the reaction of the fully protected ribonucleotide triester LXXVIII with Reagent I[188] gave after 30 min, 85.7% of the diester LXXIX along with 14.3% of chain cleavage illustrated by the presence of uridine (LXXXb) (Scheme XVIII). By using Reagent II in the last experiment, 76.5% of LXXIX and 8.6% of chain cleavage were observed after 24 hr. 14.8% of mmtUp(TCE)U remained unreacted.

3. Attempted Deprotection of a "Charged Trinucleotide" by Fluoride Ions in Dry THF

We observed, before the appearance of the recent paper[189] by Reese, that Reagent I was not really adequate for polynucleotide deprotection although certain workers reported that it worked very well for the deprotection of a hexanucleotide with less than 5% contamination by shorter oligomers[190].

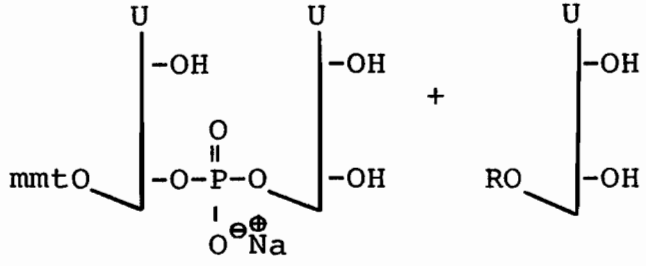
Reese and coworkers[189] found that Reagent II gave better deprotection of tetranucleotide triesters compared to Reagent I. However, caution must be exercised in projecting the use of either of these reagents for deprotecting very long chains. We have found that if one of the internucleotide units is charged, it becomes very difficult to subsequently remove the

Scheme XVIII



LXXVIII

1. Reagent I or II
2. $Na^{\oplus}$ resin column



LXXIX

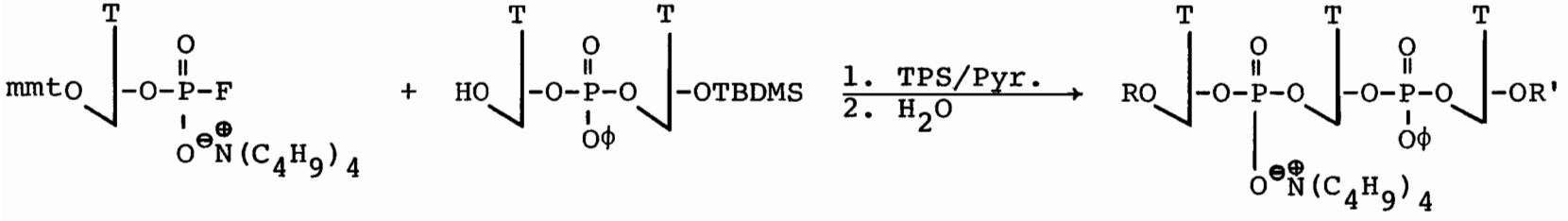
LXXXa, R=mmt
b, R=H

phenyl group from an adjacent triester.

The trinucleotide LXXXIa was synthesized from the 5'-O-p-monomethoxytrityl derivative of LXXIII with the 5'-unblocked dinucleotide LXXVId via a TPS assisted condensation (Scheme XIX). The trinucleotide LXXXIb was obtained in 69% yield as determined spectrophotometrically based on LXXVId.

Treatment of LXXXIb with TBAF (ten molar equivalents) in dry THF for 8 hr at room temperature failed to give the desired trinucleotide TpTpT. Only starting material was observed on TLC paper (Rf^A = 0.50) and electrophoresis (E_m = 0.25). LXXXIb was then treated with a 0.2 N sodium hydroxide solution for 14 hr at room temperature. Both TLC paper and electrophoresis showed the presence of TpTpT (Rf^A = 0.17, E_m = 0.62) as major product. No LXXXIb was left unreacted. Problems might arise with Reagent II in polynucleotide deprotection because it contains water which might hydrolyze a phosphorofluoridate moiety. As soon as a charge is formed on a nucleotide chain, it seems very hard to remove the adjacent phosphate protecting group. Furthermore, according to the results described earlier we do not think that either Reagent I or Reagent II will be suitable for the deprotection of polynucleotide triesters having phenyl or trichloroethyl phosphate protecting groups because in both cases the amount of internucleotide bond breaking is too high. Oximate ions[189] seem to offer good promise for the deprotection of polynucleotides

Scheme XIX



LXXVId

LXXXI a, R=mmt, R'=TBDMS
b, R=R'=H

but this new procedure is still under investigation.

Nevertheless, if it becomes possible to reduce the activity of fluoride ions by adding to them anhydrous solvating agents such as acetic acid or certain alcohols, one might hope to find new utility for this reagent.

Transesterification Reactions Induced by Fluoride Ion on Triaryl, Trialkyl and Nucleotide Phosphotriesters

1. Fluoride Ion Promoted Transesterification on Triaryl and Trialkyl Phosphotriesters

As discussed in the two last sections, protic solvents such as water or acetic acid reduced the nucleophilicity of fluoride ion. It was observed also that fluorides in alcohols such as methanol and propan-2-ol, required 25-30 hr to cleave completely the silyl protecting groups in 3',5-di-O-TBDMS thymidine. It occurred to us that regardless of the specific mechanism of the reaction, the net result of a reaction between a phenyl or trichloroethyl phosphotriester and fluoride ion in alcohol solution might be the replacement of phenoxy- or trichloroethoxy - by an alkoxy-group (from the alcohol). Indeed the reaction of triphenylphosphate with alcohols[191] such as methanol, ethanol, n-propanol, propan-2-ol, n-butanol and n-pentanol in the presence of cesium fluoride (CsF) (ten molar equivalents) produced within 8 hr the corresponding trialkyl-phosphates in virtually quantitative yields (g.l.c.) according to equation 2.

$$(\phi O)_3P{=}O \xrightarrow[ROH]{CsF} (RO)_3P{=}O + 3\phi OH \qquad \text{(Eq. 2)}$$

However, when tributyl phosphate was stirred with methanol in the presence of CsF (twenty-five molar equivalents), no exchange reaction occurred at room temperature after 24 hr. Even at 80°C for 20 hr no exchange occurred; only tributyl phosphate was detected by gas chromatography.

Among the alcohols studied for the fluoride promoted transesterification of triphenyl phosphate, a few of them such as tert-butyl alcohol, 2-cyanoethanol and 2-trimethylsilylethanol were exceptional. None of them produced the expected phosphotriesters even after 30 hr. NMR of the reaction mixture (Fig. I) containing 2-cyanoethanol as solvent showed the presence of acrylonitrile suggesting that transesterification occurred and that fluoride ion induced β-elimination producing acrylonitrile and phosphodiesters.

The encouraging results (Eq. 2) prompted us to react tris-trichloroethyl phosphate LXXXVIII with different alcohols in the presence of CsF in order to obtain mixed trialkyl-phosphates. Since these syntheses worked quite well[193], we also directed our attention to the synthesis of mixed dialkyl phenylphosphonates and cyclic phosphates (Scheme XX).

When the bis-trichloroethyl ester of phenylphosphonate (LXXXII) was stirred with absolute ethanol in the presence of CsF (ten molar equivalents) the optimum yield of the mixed alkyl phenylphosphonate LXXXIII was obtained after 19.5 hr at ambient temperature (87.7%). 7.4% of the symmetrical

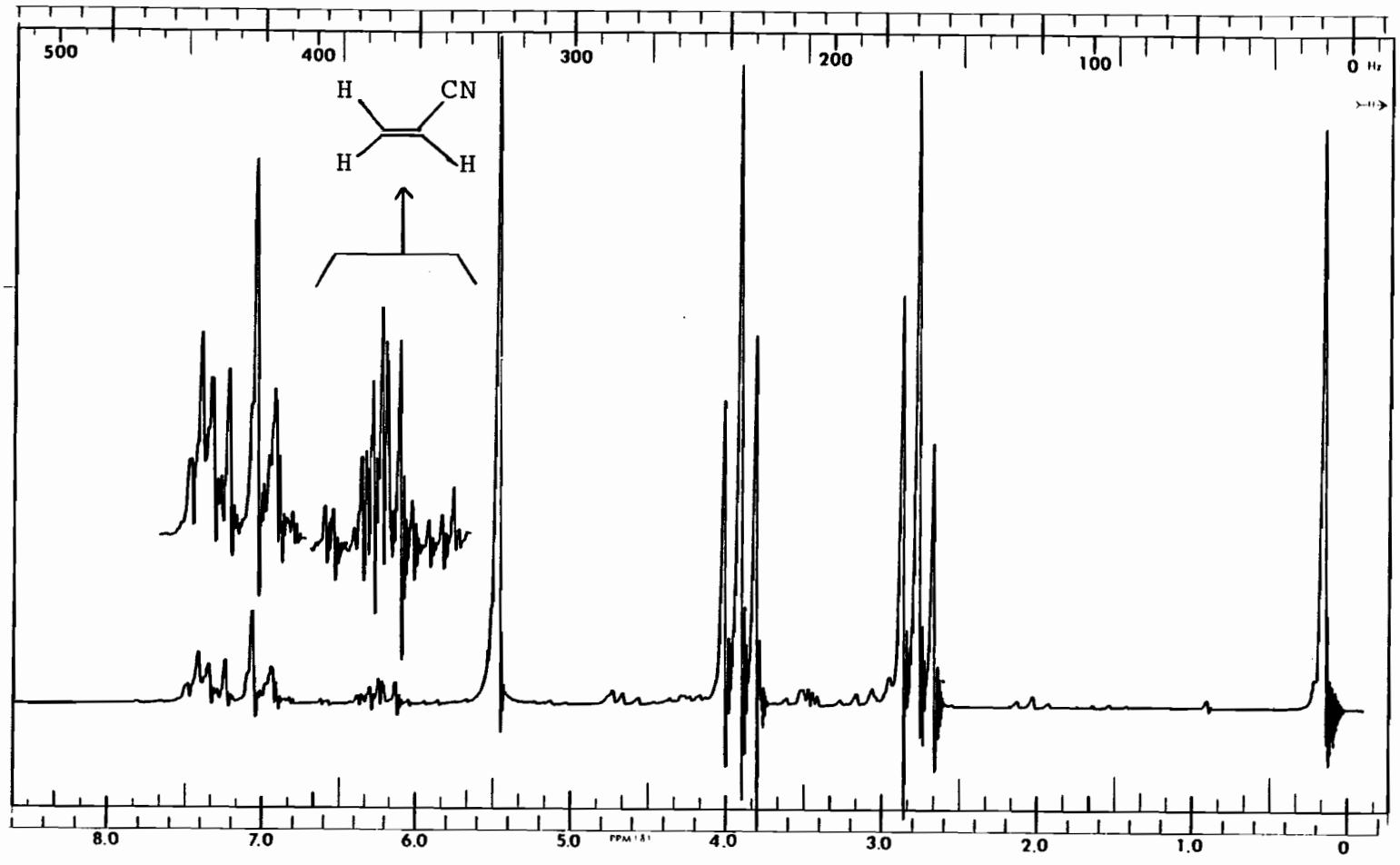
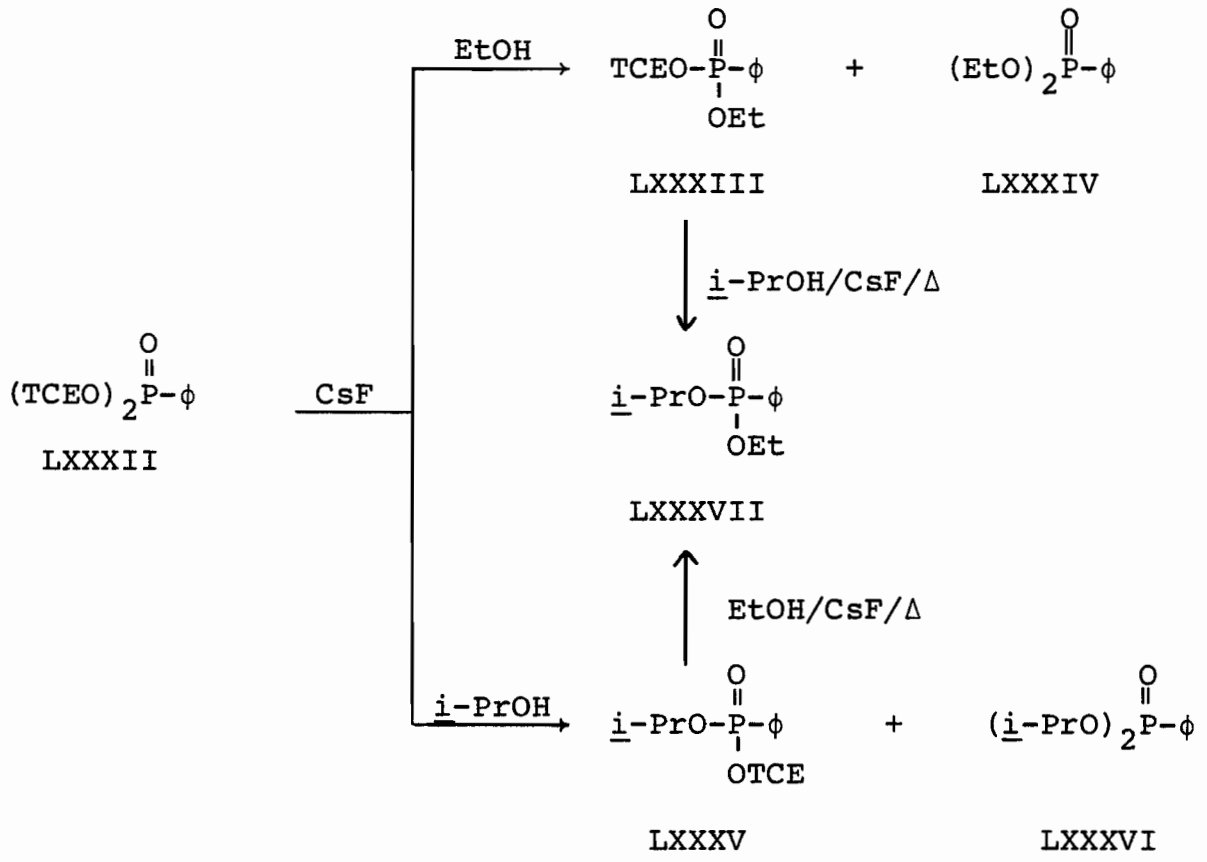
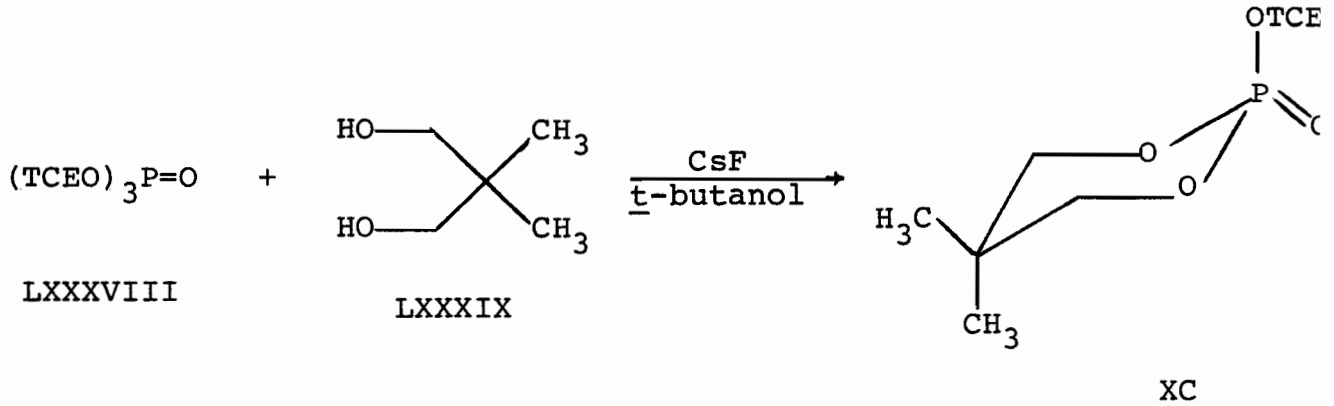


Fig. I. PMR of the Reaction of Triphenylphosphate with Cesium Fluoride in Hydracrylonitrile (2-Cyanoethanol).

Scheme XX


EtOH
TCEO-P(=O)(OEt)-φ
(EtO)2P(=O)-φ
LXXXIII
LXXXIV
i-PrOH/CsF/Δ
(TCEO)2P(=O)-φ
CsF
i-PrO-P(=O)(OEt)-φ
LXXXII
LXXXVII
EtOH/CsF/Δ
i-PrOH
i-PrO-P(=O)(OTCE)-φ
(i-PrO)2P(=O)-φ
LXXXV
LXXXVI


(TCEO)3P=O
LXXXVIII
HO
CH3
HO
CH3
LXXXIX
CsF
t-butanol
OTCE
P
O
O
H3C
CH3
XC

phosphonate LXXXIV was formed and 4.8% of unreacted starting material was left over as shown by g.l.c. LXXXIII was isolated from TLC silica gel plates in 51% yield. This was characterized by its PMR spectrum which is quite straightforward (see Fig. II and experimental section). Under similar conditions, LXXXII was stirred with dry iso-propanol in the presence of CsF. According to g.l.c. the optimum yield of the mixed alkyl phenylphosphonate LXXXV was obtained after 22.5 hr at room temperature (95.8%). Only 2.3% of the symmetrical phosphonate LXXXVI was formed and a little starting material (1.9%) remained. LXXXV was isolated from TLC plates in 59% yield and was characterized by PMR (see Fig. III and experimental section).

Finally, LXXXIII was stirred with dry iso-propanol at 75°C in the presence of CsF (five molar equivalents) for 10 hr at which time g.l.c. showed a nearly quantitative formation of the phosphonate LXXXVII (97.4%).

Similarly, LXXXV was stirred with absolute ethanol under identical conditions. After 10 hr, g.l.c. showed a 97.7% conversion of LXXXV to LXXXVII. In each case a PMR spectrum of the isolated phosphonate was taken. The two spectra were perfectly identical (see Fig. IV and experimental section). The mass spectrum of LXXXVII showed a parent peak at m/e = 228. It is important to note that in the absence of CsF, no transesterification reaction occurred at all.

The versatility of the fluoride assisted transesterification

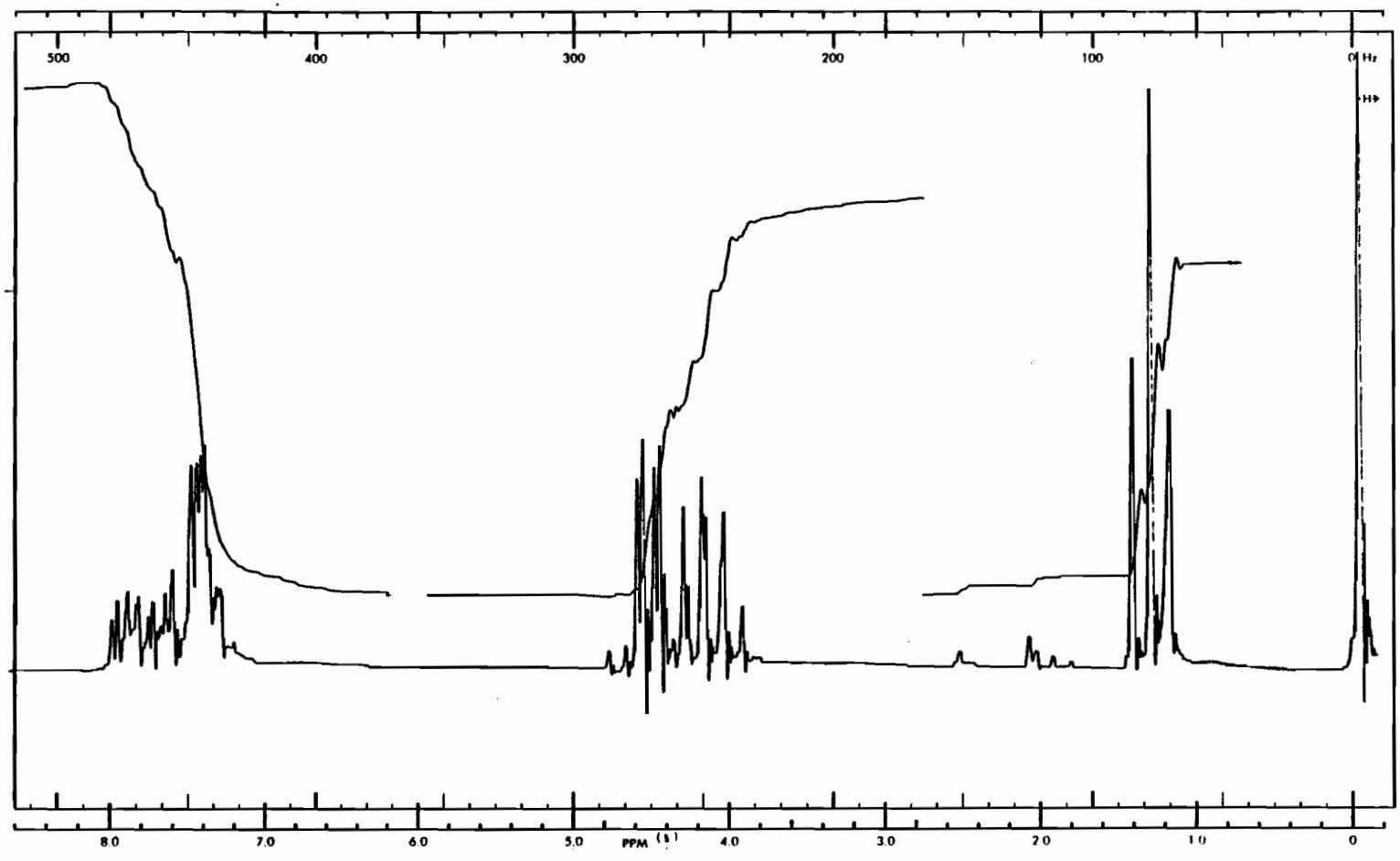


Fig. II. PMR of Ethyl, (2,2,2-Trichloroethyl), Phenylphosphonate (LXXXIII).

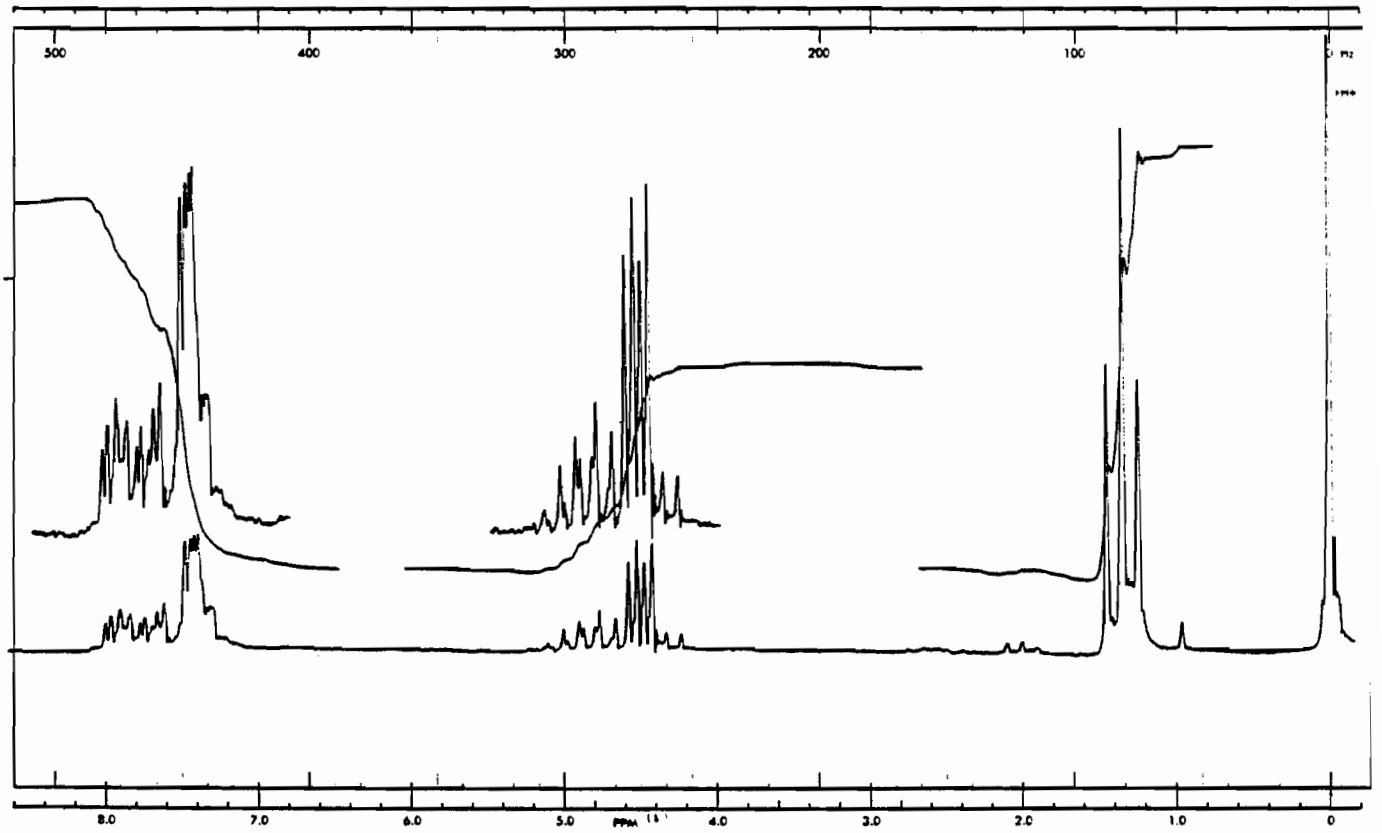


Fig. III. PMR of iso-Propyl, (2,2,2-Trichloroethyl), Phenylphosphonate (LXXXV).

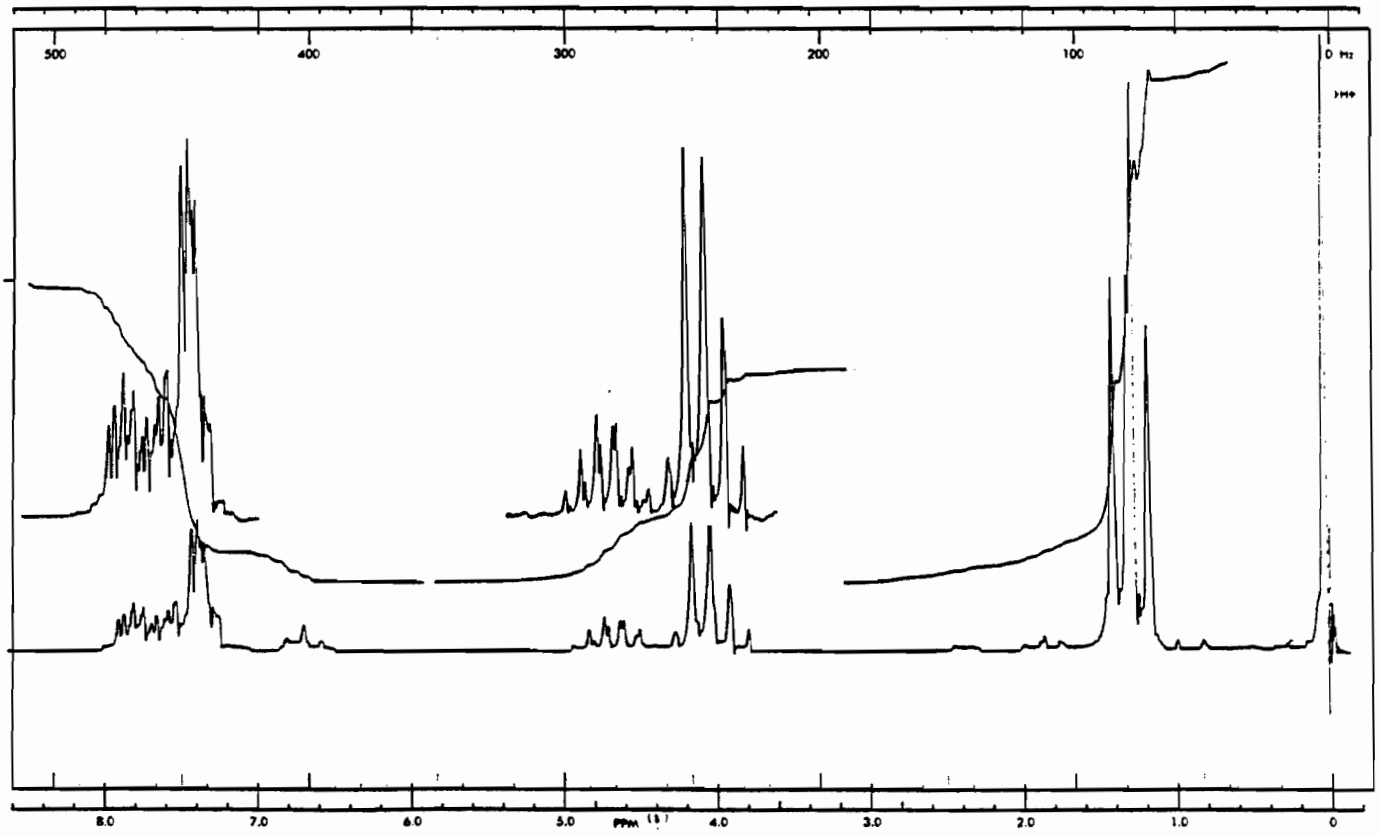
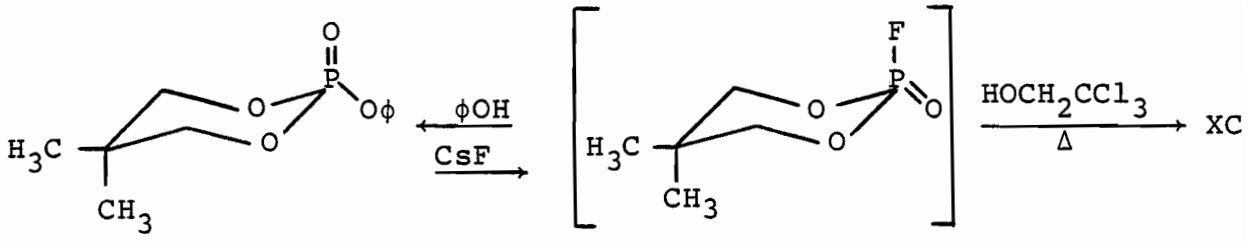


Fig. IV. PMR of Ethyl, iso-Propyl, Phenylphosphonate (LXXXVII).

reaction permits the use of solid alcohols. Since tert-butyl alcohol was found to be unreactive under the reaction conditions, it can serve as solvent for solid alcohols. This was illustrated by the synthesis of the cyclic phosphate XC (Scheme XX). tris-Trichloroethyl phosphate LXXXVIII, 2,2-dimethyl-1,3-propanediol (LXXXIX) and CsF were stirred in tert-butanol at room temperature for 20 hr. As shown by g.l.c., the cyclic phosphate XC was formed in nearly quantitative yield taking into account the amount of LXXXVIII remaining. XC was characterized by PMR (see Fig. V and experimental section). The mass spectrum displayed a parent peak at m/e = 297. Furthermore, the structure of XC was confirmed by chemical synthesis via another route. The 1,3,2-dioxaphosphorinane XCI was converted into XC in high yield according to g.l.c. by treatment with 2,2,2-trichloroethanol in the presence of CsF (five molar equivalents) after two days at 80°C. The presence of a new peak was also observed, probably corresponding to phosphorofluoridate XCII (Eq. 3).



XCI XCII (Eq. 3)

This new peak was observed also when XC and XCI were treated with

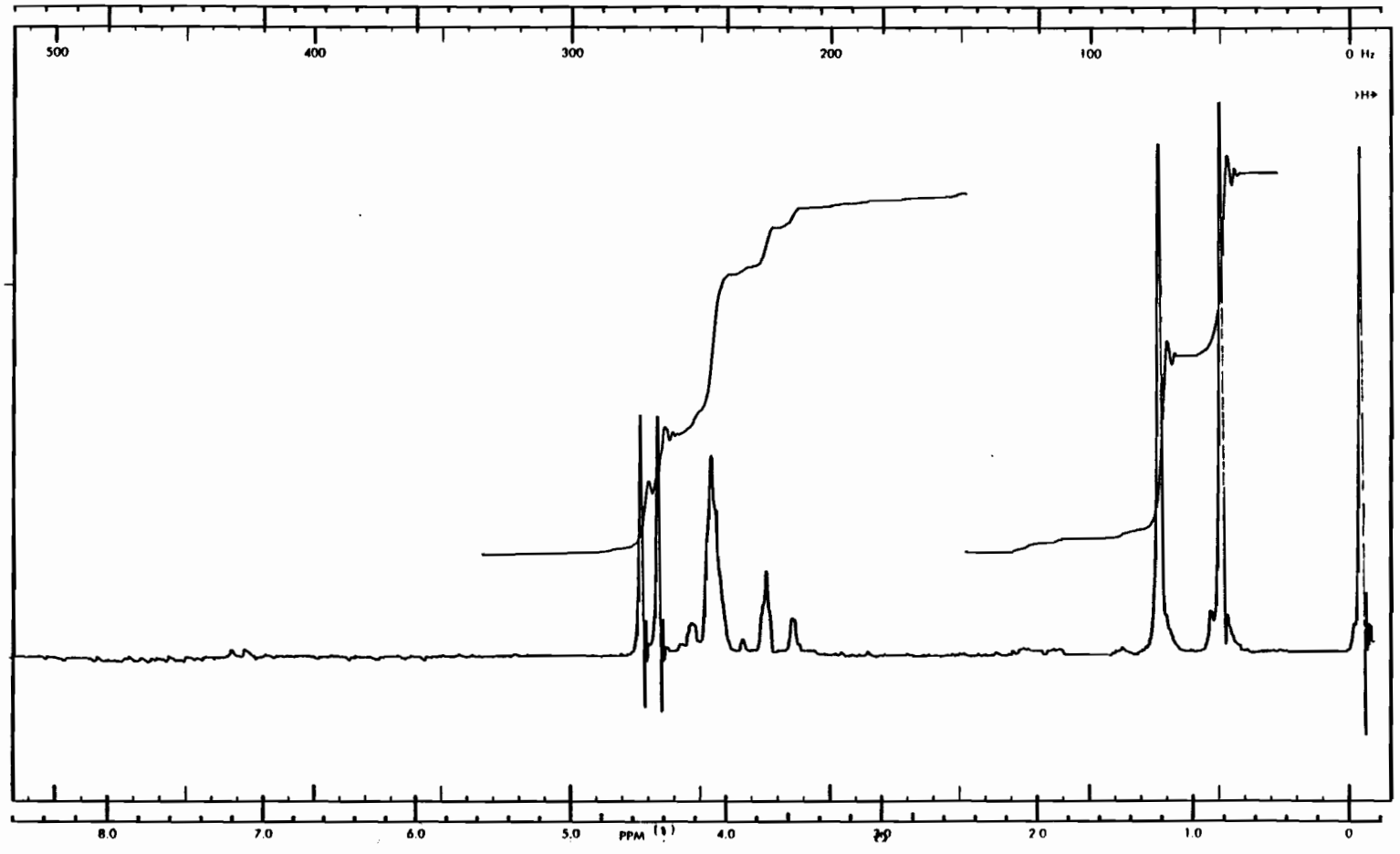


Fig. V. PMR of 5,5-Dimethyl-2-oxo-2-(2,2,2-Trichloroethoxy)-1,3,2-Dioxaphosphorinane (XC).

CsF (ten molar equivalents) in dry acetonitrile (MeCN) at room temperature for 15 hr. Figure VI shows the chromatograms of the two reactions. In both cases, the new peak appearing at lower retention time with respect to starting material corresponds probably to XCII. Furthermore, when the reaction mixtures were not stirred for a while (ca. 30 min) g.l.c. showed that the intensity of the peak corresponding to XCII decreased. Interestingly when the reaction mixtures, after a little rest, were stirred again for 60 min, g.l.c. exhibited an increase in the intensity of the peak corresponding to XCII. These observations suggest that XCII is an unstable species which is involved in the equilibrium of the reaction. When acetonitrile was replaced by a protic solvent such as methanol, ethanol or iso-propanol, g.l.c. showed the formation of the transesterification product as reported[193].

In order to check the generality of this new fluoride ion assisted transesterification, it was applied to the synthesis of thiophosphate.

Interestingly, when triphenylthiophosphate XCIII was stirred with absolute ethanol in the presence of CsF, the transesterification reaction was relatively slow and showed some selectivity according to equation 4.

$$\underset{\text{XCIII}}{(\phi O)_3P{=}S} \xrightarrow[EtOH]{CsF} \underset{\text{XCIV}}{(\phi O)_2\overset{S}{\overset{\|}{P}}\text{-OEt}} + \underset{\text{XCV}}{(EtO)_2\overset{S}{\overset{\|}{P}}\text{-O}\phi} + \underset{\text{XCVI}}{(EtO)_3P{=}S} \qquad \text{(Eq. 4)}$$

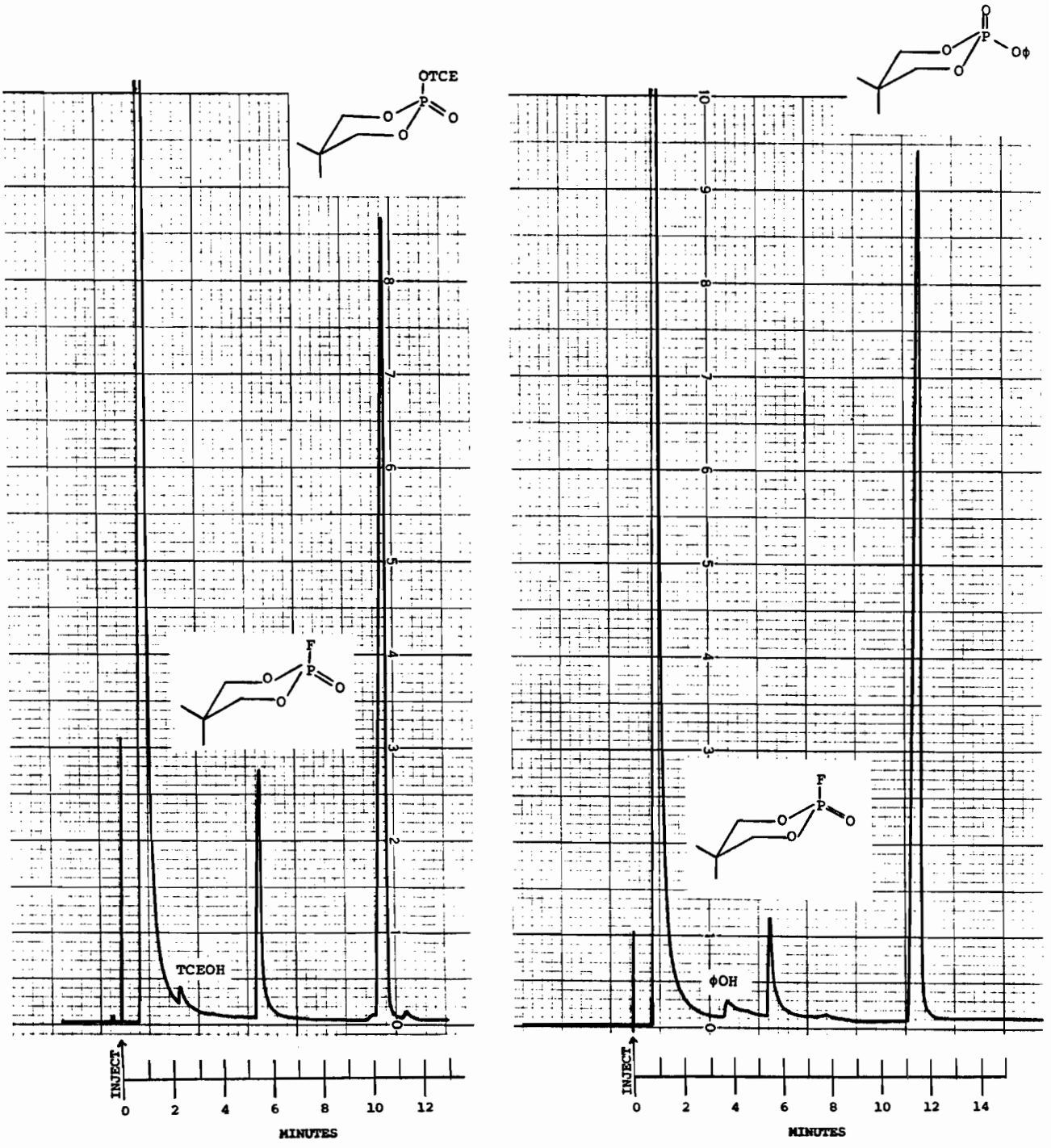
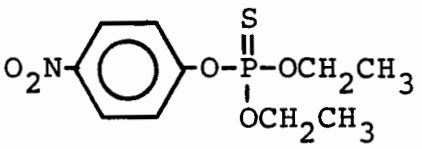


Fig. VI. Chromatograms of the Reaction of Certain Cyclic Phosphate Triesters with Cesium Fluoride in Dry Acetonitrile.

Using five molar equivalents of CsF at room temperature, after 14 hr, gas chromatography revealed the formation of 63.4% of the mono-alkyl derivative XCIV along with 14.8% of XCV and 11.7% of XCVI. 10.1% of starting material was still present. Using the same amount of CsF and heating the reaction mixture at 50°C, we observed after 23 hr the formation of 60.0% of XCIV along with 16.2% of XCV and 15.4% of XCVI. 8.4% of XCIII was left over. In a similar reaction using ten molar equivalents of CsF we observed after 23 hr at 50°C the formation of 45.4% of XCV, an analog of parathion (XCVII). XCIV and XCVI were formed in yields of 16.2% and 38.4% respectively.



XCVII

tris-Trichloroethyl thiophosphate XCVIII when stirred with absolute ethanol (Eq. 5) in the presence of CsF (ten molar equivalents) at 65°C, showed an excellent selectivity in the replacement of the trichloroethyl phosphate protecting group. After 16 hr, 87.7% of the thiophosphate XCIXa was formed along with 10.0% of Ca. Only 2.2% of starting material remained as indicated by g.l.c. Under more vigorous conditions (38 hr at

85°C) g.l.c. showed the selective formation of Ca (83.3%) along with some unwanted CIa (16.6%).

$$(TCEO)_3P{=}S \xrightarrow[ROH]{CsF} (TCEO)_2\overset{\overset{S}{\|}}{P}\text{-}OR + (RO)_2\overset{\overset{S}{\|}}{P}\text{-}OTCE + (RO)_3P{=}S \qquad \text{(Eq. 5)}$$

XCVIII XCIX a, R=Et Ca,b CIa,b
b, R=n-Pr

Similarly with n-propanol (Eq. 5) as solvent, the thiophosphate XCIXb (81.8%) was preferentially formed, according to g.l.c. after 14 hr at 65°C. 13.7% of Cb and 2.1% of CIb were also formed under these conditions. 2.4% of unreacted starting material was observed. On the other hand, Cb was present in high yield (81.4%) after 38 hr at 85°C along with 14.1% of CIb and 4.4% of XCIXb. Triphenyl phosphite, when stirred with alcohols at high temperature, underwent transesterification to some extent[192]. However, in the presence of CsF, triphenyl phosphite was converted in virtually quantitative yields to the corresponding trialkyl phosphites within 9 hr at ambient temperature as shown in equation 6. The alcohols tested include n-propanol, n-butanol and n-pentanol.

$$(\phi O)_3P: \xrightarrow[ROH]{CsF} (RO)_3P: + 3\phi OH \qquad \text{(Eq. 6)}$$

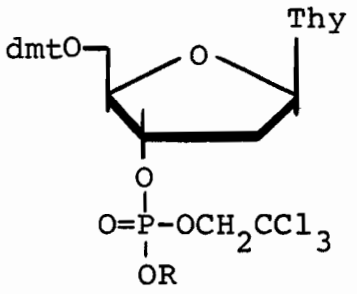
In light of the results obtained above it became of interest

to extend this transesterification reaction to mononucleotide and dinucleotide triesters.

2. Fluoride Ion Promoted Transesterification on Nucleotide Phosphotriesters

The reaction of $mmtTp^{\phi}_{\phi}$ (LXXIIa) with alcohols such as methanol, ethanol and n-butanol in the presence of anhydrous cesium fluoride (CsF) at room temperature for 8-48 hr gave the corresponding dialkyl esters $mmtTp^{Me}_{Me}$, $mmtTp^{Et}_{Et}$ and $mmtTp^{Bu}_{Bu}$ in yields of 86%, 88% and 88% respectively. Alcohols bearing functional groups on the alkyl chain such as 2-chloroethanol can also be used for transesterification reactions. Using the same conditions as above, $mmtTp^{CH_2CH_2Cl}_{CH_2CH_2Cl}$ was isolated in 84% yield.

Similarly, we treated $dmtTp^{TCE}_{TCE}$ with methanol, ethanol and n-butanol in the presence of CsF (ten molar equivalents). With methanol and ethanol as solvents, the transesterification reactions were carried out at 50-55°C. After 17.5 hr and 8.5 hr respectively the asymmetric nucleotide triesters CIIa and CIIb were isolated in 71% and 75% yield respectively. When n-butanol was used as solvent, the transesterification reaction was stirred at ambient temperature for 48 hr. The triester CIIc was isolated in 75% yield.



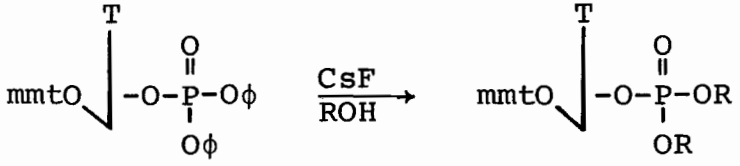
CII a, R=$-CH_3$
b, R=$-CH_2CH_3$
c, R=$-(CH_2)_3CH_3$

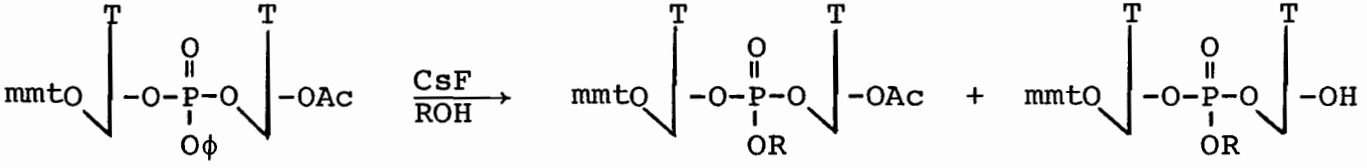
CIIa-c were detritylated according to the usual procedure to enable mass spectral analysis. The mass spectra of Tp_{Me}^{TCE}, Tp_{Et}^{TCE} and Tp_{Bu}^{TCE} showed parent peaks at m/e = 466, m/e = 480 and m/e = 508 respectively. Chromatographic and spectral data of the transesterification products are given in Table XI.

In the same vein, the dinucleotide mmtTp(ϕ)TAc was reacted with methanol, ethanol, n-butanol and CsF at room temperature. Within 48 hr the monoalkyl triesters mmtTp(Me)TAc, mmtTp(Et)TAc and mmtTp(Bu)TAc (Scheme XXI) were isolated in respective yields of 84%, 79% and 70%. Generally, we observed during the course of the reaction a loss of 7-8% of the 3'-O-acetyl protecting group. Therefore, the total yields of the transesterified products became 91%, 86% and 78% respectively. Chromatographic and spectral properties of transesterification products are listed in Table XI.

Dinucleotides bearing a trichloroethyl phosphate protecting

Scheme XXI





R = Me, Et, Bu

group were not as suitable for transesterification reactions because at room temperature it required too much time (>>100 hr) to produce a good yield of transesterified product (50-75%). For this reason these compounds were not used in the deprotection reactions described below.

3. The Use of tert-Butanol, 2-Cyanoethanol and 2-Trimethylsilyl-Ethanol in the Deprotection of Nucleotide Triesters

The phosphotriesters used in the deprotection study include dmt Tp^{ϕ}_{ϕ}, dmt Tp^{TCE}_{TCE} and mmtTp(ϕ)TSi (LXXVIa). These were stirred with each of the "special" alcohols in the presence of CsF (ten molar equivalents) at room temperature for 30-50 hr (see experimental section). After work-up, the reaction products were conventionally detritylated and were then applied to preparative paper chromatography. The yields of the bands were recorded spectrophotometrically and the results are reported in Table II. tert-Butanol and 2-trimethylsilylethanol appeared to be the best solvents for the deprotection of LXXVIa affording TpT in over 80% with < 10% yield of thymidine. The deprotection reaction seems to be slow in 2-cyanoethanol; 31% of the starting material remaining after 50 hr. In the deprotection reaction of dmtTp^{ϕ}_{ϕ}, the employment of tert-butanol gave a high removal of both phenyl phosphate protecting groups leaving just 5.5% of the phenyl ester of thymidine-3'-phosphate (Tp^{ϕ}_{OH}, LXXIVa). Consequently, this reaction becomes an

Table II. Deprotection of Mono- and Dinucleotide Triesters by Fluoride Ions* in Protic Solvents.

Starting Material	Solvent	Time (hr)	O.D. units (Product**, % yield)			
$dmtTp^{\phi}_{\phi}$	$\underline{t}$-C_4H_9OH	30	5.06 (Tp^{ϕ}_{OH}, 5.5)	62.0 (Tp^{F}_{OH}, 67.9)	21.2 (Fast moving†, 23.2)	3.00 (Others, 3.3)
$dmtTp^{\phi}_{\phi}$	$HOCH_2CH_2Si(CH_3)_3$	30	31.5 (Tp^{ϕ}_{OH}, 29.4)	35.2 (Tp^{F}_{OH}, 32.9)	29.4 (Tp, 27.5)	10.9 (T, 10.2)
$dmtTp^{\phi}_{\phi}$	$HOCH_2CH_2CN$	30	25.8 (Tp^{ϕ}_{OH}, 42.6)	9.2 (Tp^{F}_{OH}, 15.2)	15.9 (Tp^{CE}_{OH}, 26.3)	9.55 (Fast moving†, 15.8)
$dmtTp^{TCE}_{TCE}$	$\underline{t}$-C_4H_9OH	50	66.6 (Tp^{TCE}_{OH}, 73.5)	8.8 (Tp^{F}_{OH}, 9.7)	15.2 (Fast moving†, 16.8)	
$dmtTp^{TCE}_{TCE}$	$HOCH_2CH_2Si(CH_3)_3$	50	78.9 (Tp^{TCE}_{OH}, 89.8)	9.00 (T, 10.2)		
$dmtTp^{TCE}_{TCE}$	$HOCH_2CH_2CN$	50	21.1 (Tp^{TCE}_{OH}, 64.5)	11.6 (Fast moving†, 35.5)		
mmtTp(ϕ)Tsi(LXXVIa)	$\underline{t}$-C_4H_9OH	50	41.0 (TpT, 78.1)	4.01 (T, 7.6)	3.45 (Fast moving, 6.6)	
mmtTp(ϕ)TSi(LXXVIa)	$HOCH_2CH_2Si(CH_3)_3$	50	42.3 (TpT, 82.0)	9.3 (Fast moving†, 18.0)		
mmtTp(ϕ)TSi(LXXVIa)	$HOCH_2CH_2CN$	50	25.5 (TpT, 68.7)	11.6 (Fast moving†, 31.3)		

*Ten molar equivalents of cesium fluoride was used at ambient temperature.

**Obtained after treatment with 80% aqueous acetic acid (85°C, 15-30 min) (see experimental section).

†Contains some thymidine.

efficient and facile preparation of thymidine-3'-fluorophosphate (Tp^{F}_{OH}, LXXIII). 2-Trimethylsilylethanol and 2-cyanoethanol also promoted removal of both phenyl groups but to a lesser degree than tert-butanol. This is due to the fluoride ion assisted β-elimination which quickly produces Tp^{ϕ}_{OH}. This charged diester resists fluoride attack.

The deprotection of $^{dmt}Tp^{TCE}_{TCE}$ with 2-trimethylsilylethanol as solvent is interesting. Only one of the trichloroethyl phosphate protecting groups was removed affording 90% of the trichloroethyl ester of thymidine-3'-phosphate (Tp^{TCE}_{OH}, LXXIVb) and 10% of sugar-phosphate bond breaking. In tert-butanol the selectivity in the removal of the trichloroethyl phosphate protecting group was lower as exemplified by the presence of Tp^{F}_{OH} (9.7%). When 2-cyanoethanol was used as solvent, the deprotection reaction was slow but nevertheless exhibited good selectivity.

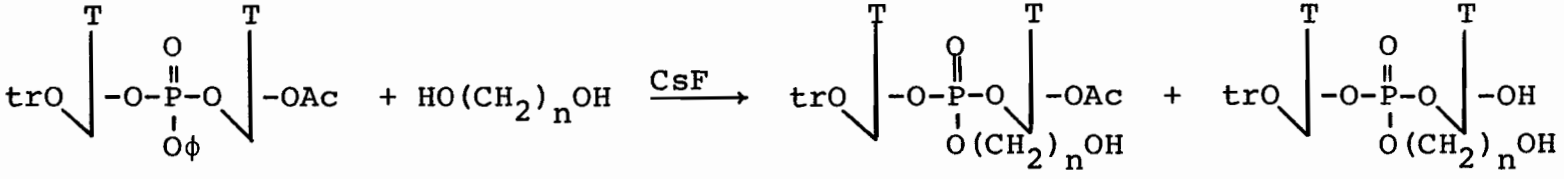
In conclusion, it was shown that the fluoride assisted transesterification reaction permitted the facile synthesis of useful phosphotriesters as well as a relatively good deprotection of LXXVIa. Tp^{F}_{OH} (LXXIII) was synthesized in high yield from this procedure and there is good reason to believe that the procedure can be extended to the synthesis of an analog of "DNA-double helix unit".

4. Synthesis of a "Phosphate Bridged" Tetranucleotide

Previous results indicate the possibility of synthesizing an analog of double stranded DNA. Rather than having two strands coupled through hydrogen bonding, it was attempted to make a model consisting of two dinucleotides, representing two strands, linked through a phosphate bridge. The strategy used in the synthesis involved the coupling of a fully protected dinucleotide bearing a hydroxy function on the phosphate protecting group such as CVa or CVb, with the fully protected dinucleotide CIII *via* a fluoride induced transesterification reaction. For this purpose, CIII was first reacted with a diol (CIVa,b) and CsF (ten molar equivalents) at room temperature for 29-48 hr (Scheme XXII).

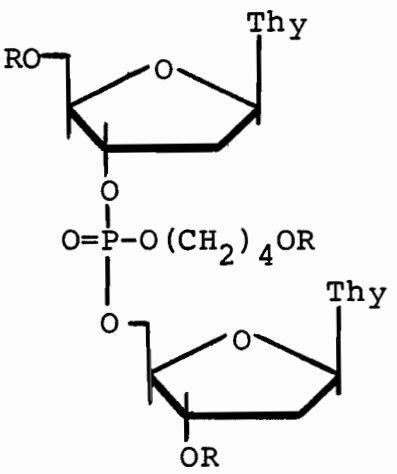
When ethylene glycol (CIVa) was used as solvent, neither CVa nor CVIa was isolated after 49 hr. Instead, trTp(ϕ)T was isolated in 17% yield along with 5'-O-trityl thymidine (trT) in 77% yield. In another run which was worked-up after 63 hr, trT was isolated in 85% yield. From these results one can suppose that CVa was formed and upon hydrogen bonding with fluoride, the β-hydroxy group assisted in the degradation of the nucleotide probably *via* a five-membered ring in the transition state. This type of degradation has also been observed by Smrt and Mikhailov[194] with analogous compounds in alkaline solution. In order to avoid this fluoride assisted

Scheme XXII

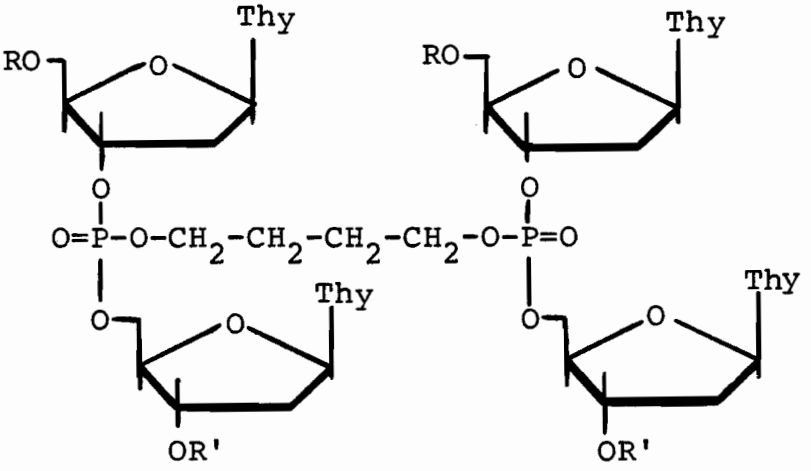


CIII CIVa, n=2 b, n=4 CVa,b CVIa,b

degradation, 1,4-butanediol was used as solvent for trans-esterification reaction. Under the conditions described for the previous case, CVb was isolated in 78% yield along with CVIb in 8% yield. CVIb was characterized by CMR as its fully deprotected form (CVIIa) and as its fully silylated form (CVIIb) (see Table III).

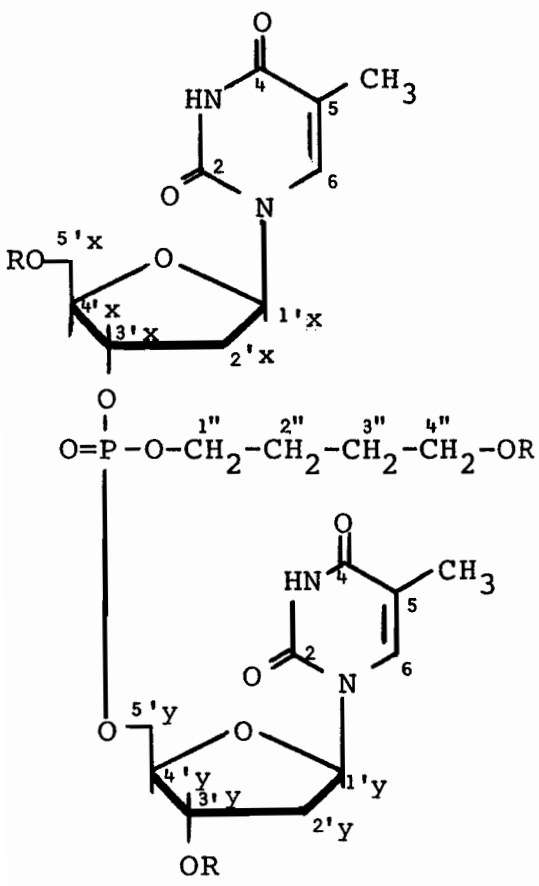


CVII a, R=H
b, R=TBDMS



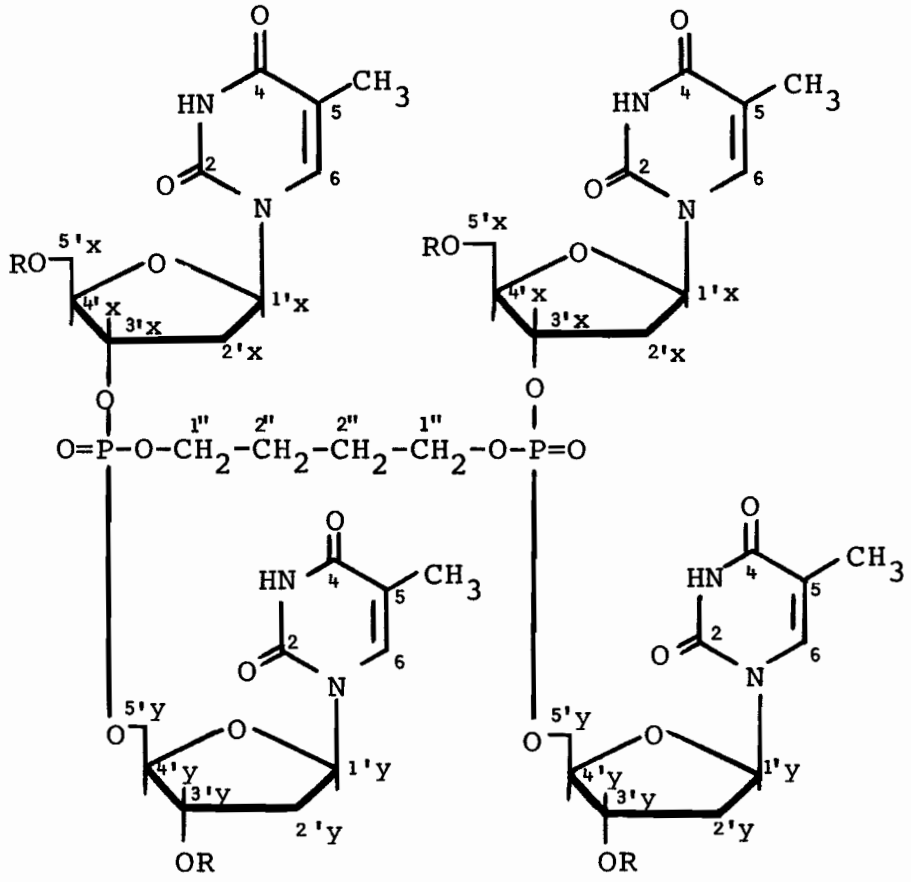
CVIII a, R=tr, R'=Ac
b, R=R'=H
c, R=R'=TBDMS

The solid alcohol CVb and the dinucleotide CIII (five molar equivalents) were dissolved in a solution of tert-butanol-N,N dimethylformamide (1:1) in the presence of CsF (ten molar equivalents). After 62 hr we isolated a mixture of starting material and a new product which moved a little faster on TLC. Attempts to cleanly separate the two materials on silica gel



CVIIa, R=H

b, R=TBDMS



CVIIIb, R=H

c, R=TBDMS

Table III. Chemical Shifts of "Bridged" Nucleotide Phosphotriesters.

Carbon	CVIIa(CD_3OD) (δ in ppm)	CVIIIb(DMSO-d-6) (δ in ppm)	CVIIb($CDCl_3$) (δ in ppm)	CVIIIc($CDCl_3$) (δ in ppm)
C-2	152.2	150.6	150.4	150.7 150.6 150.5
C-4	166.2	163.9	163.8	164.1 164.0 163.9
C-5	111.9	109.9 109.8	111.3	111.3 111.1
C-6	137.9	135.8	135.6 134.9	136.3 134.8
Me	12.6 12.5	12.2 12.0	12.5	12.5
C-1'x	86.6 ‡	84.1 ‡	85.6 85.4 ‡	86.1 85.7 ‡
C-1'y	86.1	83.8	84.9 84.8	85.1 84.8
C-2'x	39.5 39.4	*	39.5 39.2	39.5 39.4
C-2'y	40.4	*	40.7	40.3
C-3'x	80.4 80.2	78.6 78.3	79.2 78.9	79.5 79.4
C-3'y	71.7	70.0	71.5	71.5
C-4'x	87.2 86.9 ‡	85.5 85.2 ‡	86.0 85.8 ‡	86.1 85.7 ‡
C-4'y	86.1 85.8	84.5 84.1	85.6 85.4	85.1 84.8
C-5'x	62.2	61.0	62.3	63.4
C-5'y	70.0 69.8	67.3** 67.1**	68.8 68.5	67.8 67.5
C-1"	68.9 68.7	67.3** 67.1**	66.9 66.7	66.9†
C-2"	28.1 27.8	26.1 25.8	27.2 26.9	25.5 26.2
C-3"	29.5		28.7	
C-4"	62.5		63.4	

*Obscured by the solvent (DMSO-d-6).

**C-5'y and C-1" were superimposed.

†Unresolved doublet.

‡Tentative assignment.

failed. The mixture of compounds was therefore silylated according to Ogilvie's procedure[173]. The separation of the two compounds then proved to be successful. CVIIIa was isolated as a pure powder in 30% yield. A molecular weight determination (Galbraith Laboratories) was in agreement with the assigned structure (within 1%). The fully deprotected CVIIIb as well as the tetrasilyl derivative CVIIIc were characterized by CMR (see Table III). The chemical shifts for CVIIa,b and CVIIIb,c were first assigned by comparison with those obtained for thymidine in a similar solvent. In addition, the splittings arising from the long range ^{13}C-^{31}P couplings were useful in determining whether a carbon was in the 5'-end (denoted by x) or the 3'-end (denoted by y) of the molecule. For instance, in CVIIa,b and CVIIIb,c, C-2' carbons appear as a doublet and a singlet. Due to ^{13}C-^{31}P coupling it is obvious that the doublet corresponds to C-2'x while the singlet corresponds to C-2'y. It has been also observed that $|J_{CCOP}|$ is often larger than $|J_{COP}|$[238]. For example, with CVIIa $^{3}J_{(C\text{-}4'x)\text{-}P}$ is 5.86 Hz and $^{2}J_{(C\text{-}3'x)\text{-}P}$ is 4.88 Hz. With CVIIIb, $^{3}J_{(C\text{-}2")\text{-}P}$ is 6.84 Hz and $^{2}J_{(C\text{-}1")\text{-}P}$ is 4.88 Hz (Table IV).

The simple two-step synthesis of CVIII is an excellent example of the versatility of the fluoride promoted trans-esterification reaction. Notably, the coupling between the two dinucleotides occurred with relative ease considering the steric hindrance created by the approach of the two huge reagents.

Table IV. ^{13}C Coupling Constant for "Bridged" Nucleotide Phosphotriesters.

Carbon	CVIIa J(Hz)	CVIIb J(Hz)	CVIIIb J(Hz)	CVIIIc J(Hz)
C-2'x (CCOP)	2.93	5.86	*	2.04
C-3'x (COP)	4.88	5.86	4.88	3.85
C-4'x (CCOP)	5.86	4.88	5.86	9.76
C-4'y (CCOP)	6.84	4.88	9.76	7.81
C-5'y (COP)	5.86	5.86	4.88	5.86
C-1" (COP)	3.91	4.88	4.88	**
C-2" (CCOP)	6.84	6.84	6.84	7.81

*Obscured by the solvent (DMSO d-6).

**Unresolved doublet.

From the results of this section it is clear that fluoride ion is an excellent reagent in promoting exchange reactions on neutral esters of phosphoric acid. The mechanism may involve the attack of fluoride on phosphorus to produce an intermediate phosphorofluoridate which reacts with the alcohol solvent. Alternately, fluoride ion may simply hydrogen bond to the alcohol causing the oxygen of the alcohol to become much more nucleophilic, displacing the phenoxy or trichloroethoxy group directly. It is also possible that a combination of these mechanisms is involved.

The hydrogen-bonding between fluorides and alcohols should not be overlooked for the improvement of classical reactions such as acylations and alkylations. This will be dealt with in the next section.

Acylation and Alkylation of Nucleosides and Nucleotides Using Tetrabutylammonium Fluoride

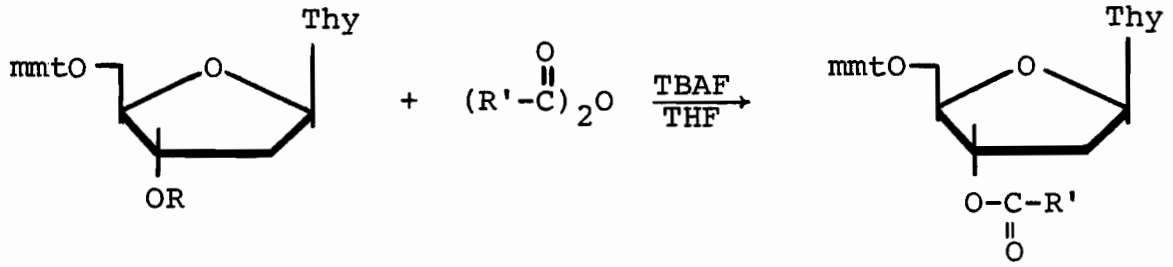
1. Acylation of Nucleosides

The routine acylation of hydroxyl groups in nucleoside chemistry usually involves an acid chloride or an acid anhydride in pyridine as solvent. These reagents often give side reactions with thymine and uracil nucleosides, presumably through reaction at the base and consequently decrease the yield of the desired O-acylated nucleosides[195-196]. Furthermore, in the case of nucleosides bearing a hindered hydroxy function, conventional acylation procedures sometimes fail to react[196].

A procedure requiring fluoride ion which gives near quantitative yields for acylation is being described using acid anhydrides with tetrahydrofuran (THF) as solvent[197]. No detectable side reactions at the nucleoside bases were observed. A variety of acid anhydrides were used in order to determine the scope of the reactions. To illustrate the effectiveness of the reagent, one should first consider the classical 3'-pivaloylation of 5'-O-p-monomethoxytrityl thymidine (LXVb) using pivalic anhydride (CIXb) in pyridine as solvent. After 24 hr at room temperature, only 9% of the 3'-pivaloylated nucleoside (CXb) was isolated. When pivalic anhydride was replaced by the more reactive pivaloyl chloride,

under similar conditions, 22% of CXb was isolated as well as 78% of a material moving faster than CXb on TLC. The latter compound was CXb with an additional pivaloyl group on the base[196].

On the other hand, when LXVb was treated with CIXb in the presence of TBAF in THF, a quantitative conversion to CXb was observed after 5 hr. No detectable amount of reaction at the base was observed even after 24 hr. In the absence of TBAF, the latter reaction produced less than 5% of CXb leaving more than 95% of starting material.



LXV b, R=H
c, R=TBDMS
d, R=mmt

CIX a, R'=-CH_3
b, R'=-$C(CH_3)_3$
c, R'=-C_6H_5
d, R'=-$(CH_2)_{12}CH_3$

CX a-d

The highest yield of acylation in the minimum of time was achieved by using ten molar equivalents of TBAF and twenty-five molar equivalents of the acid anhydride.

The classical acylation of LXVb with acetic anhydride (CIXa) in pyridine gave, after 24 hr, 80% of CXa and 20% of a

material moving much faster on TLC than CXa. Under the same conditions with benzoic anhydride (CIXc), CXc was isolated in 65% yield leaving 35% of unreacted LXVb. No reaction at the base was observed for this case. CXd was isolated in 42% yield from an analogous reaction with myristic anhydride (CIXd). No reaction at the base was observed.

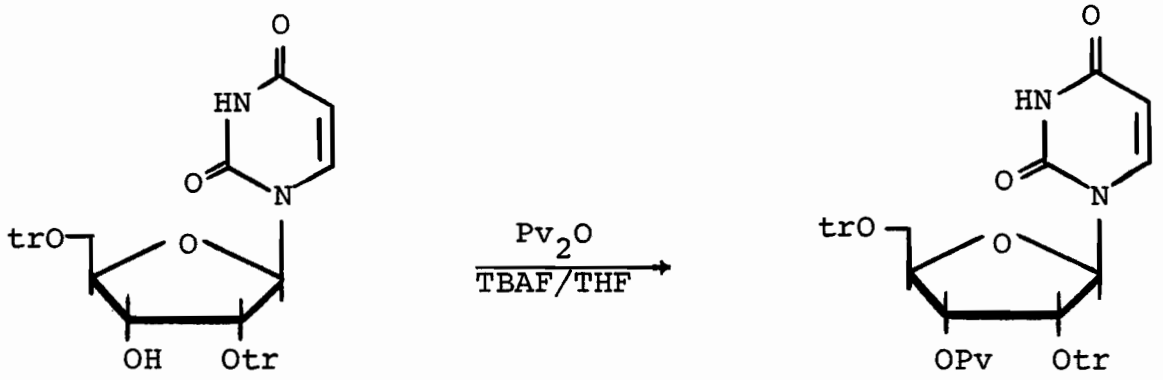
When pyridine was replaced by TBAF in THF, CXa, CXc and CXd were isolated on TLC plates in respective yields of 99% (30 min), 98% (2 hr) and 55% (8 hr). The yield of CXd attained 64% when the reaction mixture was stirred at reflux temperature for 4 hr.

Acylation at the base was nonexistent in all the cases studied. In the absence of TBAF, CXa, CXc and CXd were isolated in 8%, 36% and 0% respectively leaving unreacted starting material (LXVb) in all cases.

From the experiments described above, it was observed that no acylation occurred at the base at room temperature for extended periods of time (24 hr). The effect of heat on the product distribution was then determined by reacting thymidine with pivalic anhydride in the presence of TBAF in THF. The solution was stirred at 85°C (screw cap vial) for 24 hr. 3',5'-di-O-pivaloyl thymidine was formed in quantitative yield without any apparent reaction at the base. Its structure was confirmed by mass spectrometry (m/e = 410). In addition, the reaction of 5'-O-pivaloyl thymidine with CIXb in the

presence of TBAF in THF at either 22°C or 85°C gave after 24 hr a quantitative formation of a material which had identical thin-layer chromatographic properties as those obtained from the previous preparation.

LXVd was found to be unreactive under the conditions used previously. Therefore with CIXb as acylating agent, no reaction occurred at the base even at high temperature (85°C) for extended periods of time (24 hr). From these experiments, it became possible to demonstrate the effectiveness of this acylation procedure on sterically hindered hydroxyl groups. 2',5'-di-O-trityluridine (CXI) was treated with CIXb in the presence of TBAF in THF at 80°C for 3 hr. The fully protected nucleoside CXII was isolated in 96% yield. PMR (Fig. VII) displays a sharp singlet at 1.2 ppm which accounts for the 9 tert-butyl protons.



CXI CXII

tr = Triphenylmethyl; Pv_2O = Pivalic anhydride.

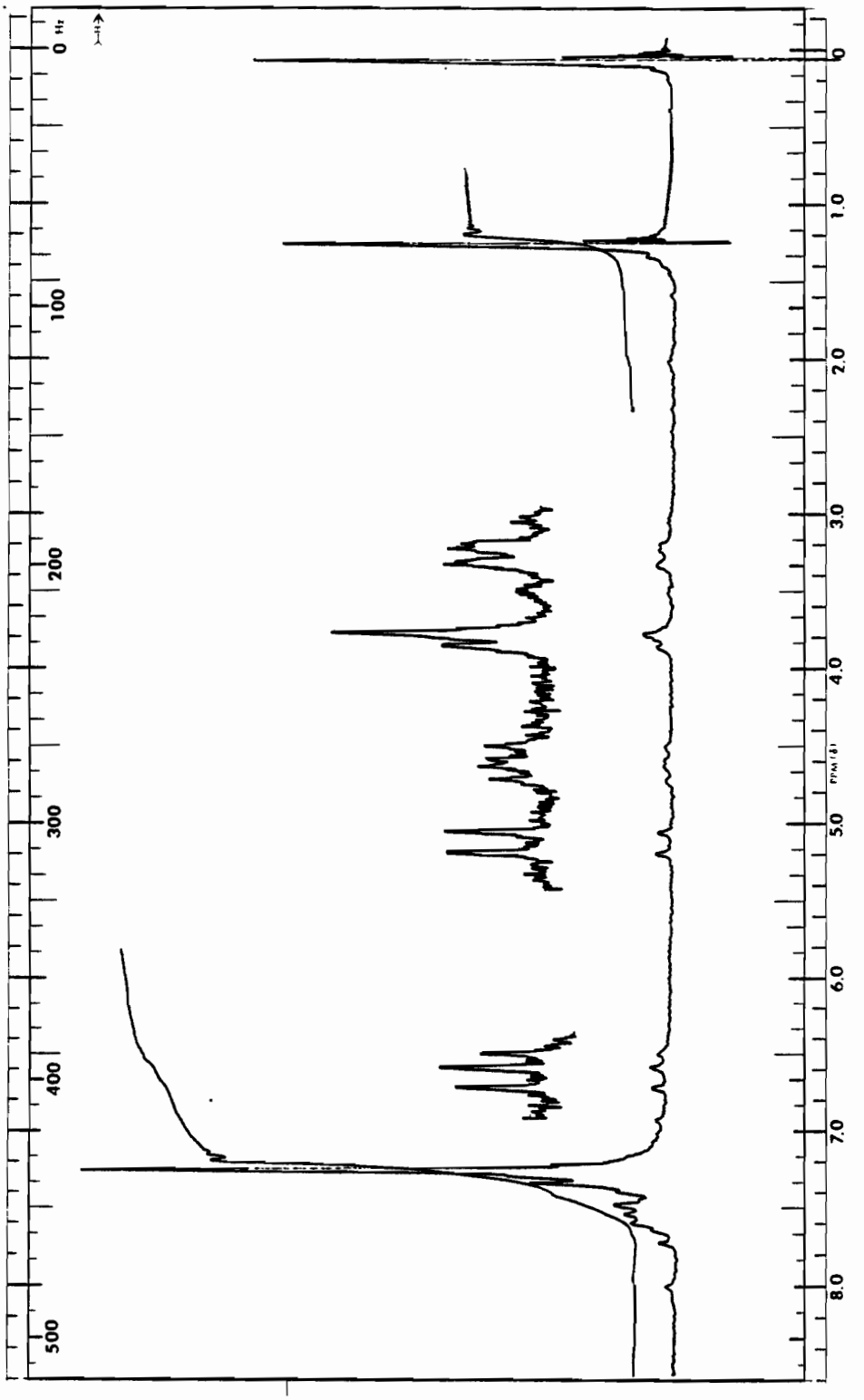


Fig. VII. PMR of 2',5'-di-O-Trityl-3'-O-Pivaloyl Uridine (CXII).

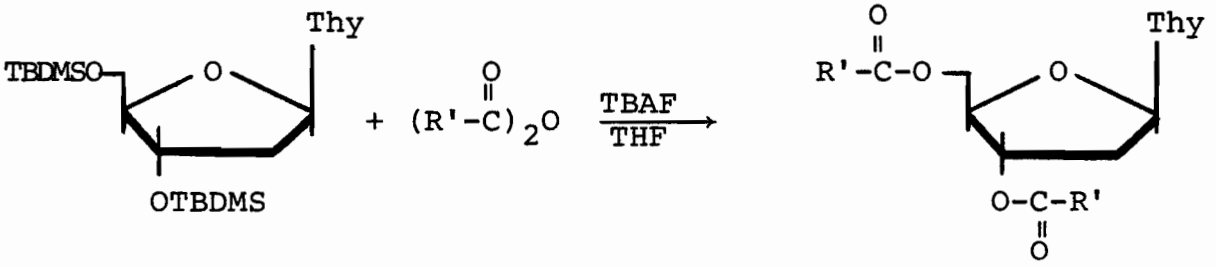
This contrasts with the previously reported[196] reaction in pyridine where no reaction occurred at the hydroxyl position with sterically hindered anhydrides.

The reaction conditions of the fluoride assisted acylation work well even for nucleosides which are not normally soluble in THF but which dissolve readily when TBAF is added. For instance, deoxycytidine was converted in 90% yield to the N-benzoyl-3',5'-di-O-benzoyldeoxycytidine after 24 hr at room temperature. Its structure was established by the classical synthesis of the compound[172]. The reaction of deoxyadenosine with CIXc in the presence of TBAF in THF after 24 hr yielded 78% of 3',5'-di-O-benzoyldeoxyadenosine (m/e = 459) and 17% of N-benzoyl-3',5'-di-O-benzoyldeoxyadenosine. Under similar conditions, deoxyguanosine gave an 81% yield of 3',5'-di-O-benzoyldeoxyguanosine.

Furthermore, the conditions used for this new acylation procedure allow the direct replacement of a silyl protecting group by an acyl group. This latter feature permits the <u>in situ</u> replacement of an alkali resistant protecting group by an alkali sensitive protecting group. A considerable flexibility is thus obtained in synthetic schemes requiring, as in nucleotide synthesis, the temporary protection of hydroxyl groups.

This aspect is exemplified by the reaction of 3',5'-di-O-TBDMS thymidine (CXIII) with twenty-five molar equivalents of CIXa-c and ten molar equivalents of TBAF in dry THF at room

temperature. Under these conditions , 3',5'-di-O-acetylthymidine (CXIVa), 3',5'-di-O-pivaloylthymidine (CXIVb) and 3',5'-di-O-benzoylthymidine (CXIVc) were isolated in 97%, 96% and 98% yield respectively after 9 hr. With myristic anhydride (CIXd) as acylating agent, 3',5'-di-O-myristoylthymidine (CXIVd) was isolated in 49% yield after 12 hr along with 51% of 5'-O-myristoylthymidine (identified by conversion to the 3'-O-TBDMS thymidine <u>via</u> silylation[173] and saponification with ammonium hydroxide, see experimental section). On the other hand, if the reaction mixture was stirred at reflux for 4 hr, an 83% yield of CXIVd was isolated along with 12% of 5'-O-myristoylthymidine and 4% of 3'-O-myristoylthymidine (m/e = 452).



CXIII CIXa-d CXIVa-d

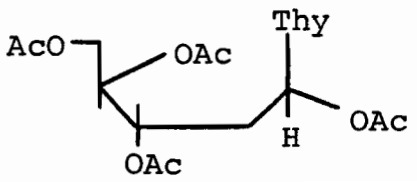
a, R'=-CH_3; b, R'=-$C(CH_3)_3$; c, R'=-C_6H_5; d, R'=-$(CH_2)_{12}CH_3$

As expected, the reaction of LXVc with CIXa-c with TBAF in THF produced CXa-c in yields ranging from 94-97% (see experimental section). LXVc was converted in the presence of TBAF in THF into CXd in 40% yield at room temperature after

15 hr. 60% of LXVb was also isolated. After 4 hr at reflux temperature, the yield of CXd increased to 48% and the yield of LXVb decreased to 52%. In the absence of TBAF, CXIII and LXVc gave no reaction with CIXa-d.

However, when CXIII was stirred with twenty-five molar equivalents of CIXa in dry pyridine for 24 hr, two products were isolated on TLC plates and their yields were recorded spectrophotometrically. The starting material CXIII was recovered in 81.5% yield. An unidentified product with a much higher mobility on TLC than CXIII was obtained in 18.5% yield. The product was not CXIVa. The reaction of LXVc with CIXa in pyridine gave rise also to the formation of a new product which moved faster than LXVc on TLC. As we have observed before, the new products from the two last reactions resulted probably from an acylation reaction at the base.

Ganem and Small[198] reported the use of ferric chloride in acetic anhydride as a mild and versatile reagent for the cleavage of ethers including tert-butyldimethylsilyl ethers. This ether-to-acetate transformation prompted us to use their conditions to convert CXIII to the diacetate CXIVa. However, instead of CXIVa we obtained the tetraacetate CXV in 76% yield as evidenced by PMR, which indicated the presence of four acetyl groups, and by mass spectrometry which showed a



CXV

parent peak at m/e = 428.

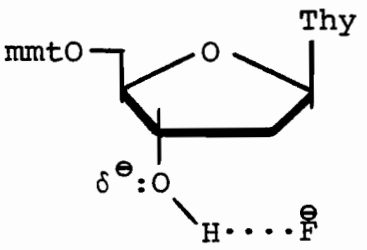
The formation of the tetraacetate (CXV) may be explained by the cleavage of the ether function of the sugar ring. This assumption is supported by the work of Goldsmith et al.[199] which relates the cleavage of cyclic ethers by magnesium bromide in acetic anhydride.

Interestingly, when CXIII was reacted with ferric chloride in pivalic anhydride, the desired 3',5'-di-O-pivaloylthymidine (CXIVb) was isolated in 62% yield without any apparent opening of the sugar ring. The structure was proved by mass spectrometry which gave a parent peak at m/e = 410. CXIVb obtained from the ferric chloride reaction was fully deprotected by treatment with a solution of sodium hydroxide (0.2 N) for 7 hr at room temperature. A material having the same mobility as thymidine was the sole nucleosidic material observed on paper chromatography using solvents A and B'. Unfortunately, the use of tritylated nucleoside is prohibited in these reactions because the presence of ferric chloride causes cleavage of the trityl ether function. Although the ether-to-ester transformation works well in pivalic anhydride, serious drawbacks are obviously encountered in acetic anhydride and with the use of tritylated nucleosides.

A decade ago, Bunton[200] showed that fluoride ion catalyzed the hydrolysis of acetic and succinic anhydrides in water and in aqueous dioxane.

For the aqueous hydrolysis of acetic anhydride it was shown that the rate of appearance of acetic acid was slower than the rate of the catalyzed disappearance of the anhydride suggesting that acetyl fluoride accumulated during the reaction.

It may be that in an analogous manner, fluoride ion reacts quickly with acid anhydride in aprotic solvent producing the acid fluoride. Subsequently, the free hydroxyl group and/or the more nucleophilic fluoride hydrogen bonded hydroxyl group (CXVI) may react with the acid fluoride to afford acylated product.

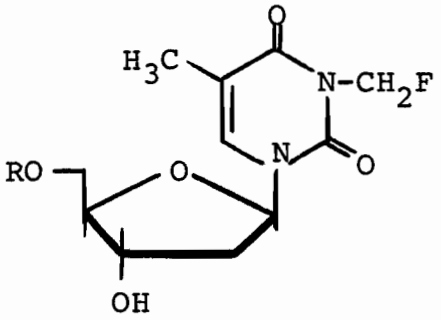


CXVI

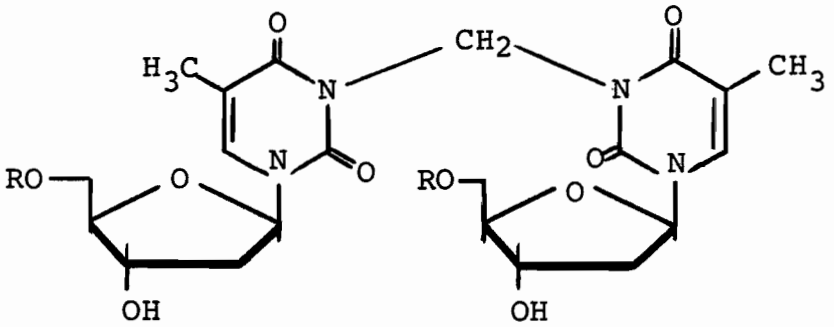
2. Alkylation of Nucleosides

Based on the previous results with acid anhydrides it was planned to substitute the anhydride by an alkyl chloride with the aim of O-alkylating nucleosides. To test the feasibility of this, LXVb was treated with five molar equivalents of TBAF using dichloromethane (CH_2Cl_2) as solvent. After extractions and purification on silica gel TLC plates, a faint band and a large band were isolated. After deprotection

with 80% aqueous acetic acid, thymidine unexpectedly moved faster on silica gel than the detritylated major reaction product. The detritylated minor reaction product moved slightly faster than thymidine on silica gel. Both reaction products were characterized by both low and high resolution mass spectrometry, PMR and CMR spectrometry. These spectra show that the structure of the minor reaction product is N^3-(fluoromethyl)thymidine (CXVIIa) and the major reaction product is di(thymidin-N^3-yl)methane (CXVIIIa).



CXVII a, R=H
b, R=mmt



CXVIII a, R=H
b, R=mmt

The protons of the N^3-CH_2 group in CXVIIa appear as a doublet ($'J_{H-F}$ = 51 Hz) at 5.5 and 6.4 ppm, split by the ^{19}F nucleus. Similarly, the CMR spectrum of CXVIIa exhibits a doublet for the N^3-C ($'J_{C-F}$ = 197 Hz) at 75.8 and 84.5 ppm with respect to tetramethylsilane (TMS) (Table V).

From mass spectral analysis it is obvious that CXVIIIa is a dimeric compound (see experimental section). The CMR spectrum exhibits an additional peak at 40.4 ppm with respect to the CMR spectrum of thymidine itself, suggesting a perfect symmetry at

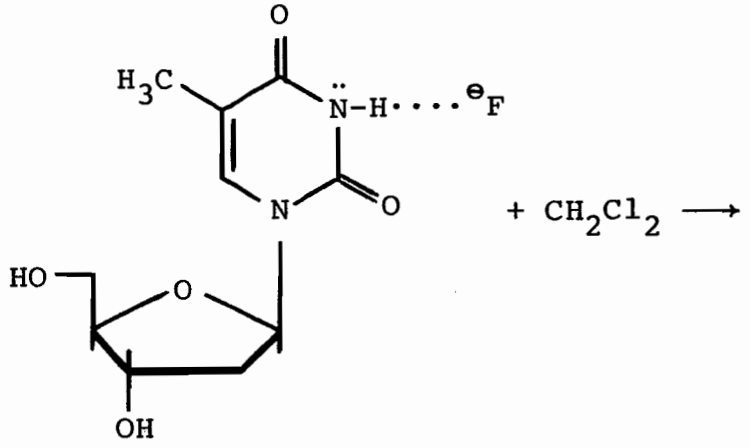
the N^3-C centre (Table V). Products CXVIIa and CXVIIIa were isolated in 8% and 91% yield respectively. Spectra showed that no alkylation occurred at the 3'-hydroxy function. When thymidine (LXIV) was stirred with TBAF in dichloromethane, CXVIIa and CXVIIIa were the sole nucleosides isolated, demonstrating that no alkylation occurred at either 3'- or 5'-hydroxy functions.

The one step "bridge" formation in dichloromethane is quite remarkable. This reaction probably involves the presence of a reactive intermediate. Scheme XXIII shows a tentative explanation of the reaction mechanism. Fluoride ion forms a strong hydrogen bond with the proton at N^3 on the base enhancing the nucleophilicity of the nitrogen which reacts with dichloromethane affording the reaction species CXIX and CXX. The latter can then react with either fluoride ion to give CXVIIa or with LXIV to give CXVIIIa.

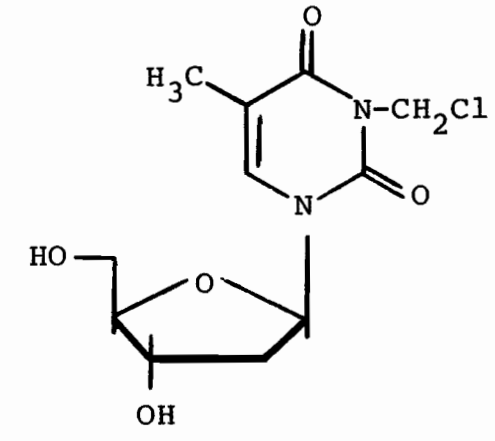
In this one step "bridge" formation, the presence of fluoride ion is essential. When TBAF was replaced by triethylamine, no reaction occurred even after several days.

Recently, nucleoside dimers bridged through their bases have generated interest. These molecules may provide information concerning the interactions that can exist between two strands of DNA or RNA, especially when portions of nucleic acid molecules do not form hydrogen bonded structures, but interact with each other through base stacking. Base stacking

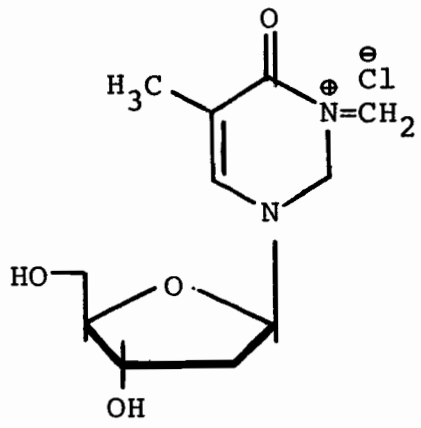
Scheme XXIII



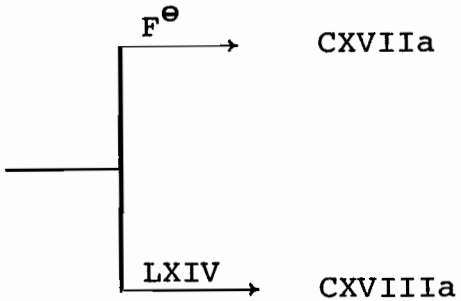
LXIV



CXIX



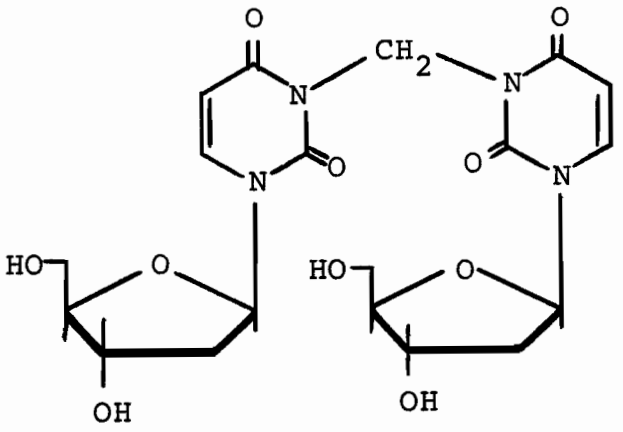
CXX



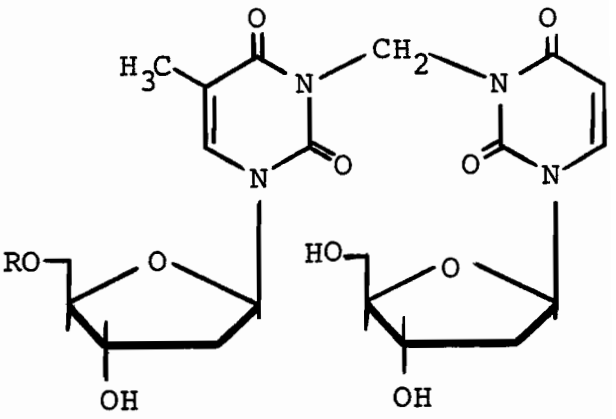
is probably responsible for the secondary structure of DNA and RNA[206]. In relation to this aspect many studies have been done on "bridged" nucleoside bases[201-205]. A few reports have been concerned with nucleosides bridged through purine bases[206-209]. Studies made with nucleosides bridged through thymine and/or uracil bases are even rarer. Therefore, the TBAF assisted alkylation reaction was applied to the synthesis of interesting bridged nucleoside derivatives.

The synthesis of di(deoxyuridin-N^3-yl)methane (CXXI) was achieved by the reaction of deoxyuridine with dichloromethane in the presence of TBAF. Before work-up, the reaction mixture was acetylated with acetic anhydride in pyridine. This enabled fully protected nucleosides to be separated from excess TBAF by a conventional extraction. The acetylated nucleosides were then deacetylated with concentrated ammonium hydroxide in pyridine. Purification on silica gel TLC plates afforded N^3-(fluoromethyl)deoxyuridine and CXXI in 14% and 84% yield respectively. CXXI was fully characterized by both low and high resolution mass spectrometry and by CMR spectrometry (Table V).

The synthesis of CXVIIIa and CXXI can be complemented by the synthesis of (deoxyuridin-N^3-yl),(thymidin-N^3-yl) methane (CXXIIa).



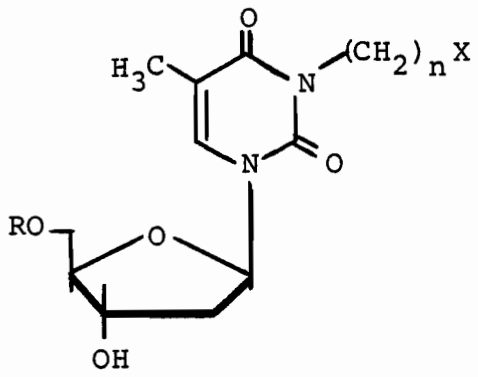
CXXI



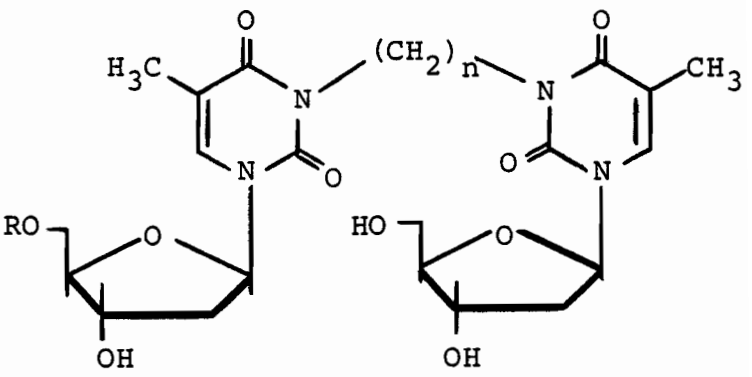
CXXII a, R=H
b, R=mmt

The latter might be difficult to obtain if thymidine and deoxyuridine were reacted with dichloromethane in the presence of TBAF, because the product mixture could be composed of an equimolar ratio of CXVIIIa, CXXI and CXXIIa, complicating the purification step on silica gel. This problem was overcome by reacting LXVb and deoxyuridine in dichloromethane in the presence of TBAF. From the three possible reaction products, CXVIIIb, CXXIIb and CXXI, the latter is readily eliminated by aqueous work-up. CXVIIIb and CXXIIb are easily separated on silica gel plates because CXVIIIb bears two p-monomethoxytrityl group while CXXIIb has only one. CXVIIIb and CXXIIb were isolated in yields of 29% and 27% respectively. These are very good yields considering that the equal reactivity of the bases restricts the maximum yield of any one dimer to 33%. CXXIIa was also characterized by low and high resolution mass spectrometry. Features of the CMR spectrum of CXXIIa are reported in Table V.

Unlike the alkylation reaction in dichloromethane, the alkylations of LXVb with 1,2-dichloroethane and with 1,6-dichlorohexane in the presence of TBAF (five or ten molar equivalents) for four to six hours at room temperature respectively gave N^3-(2-chloroethyl)5'-O-p-monomethoxytrityl thymidine (CXXIIIa) in 97% yield and N^3-(6-chlorohexyl)5'-O-p-monomethoxytrityl thymidine (CXXIIIc) in 100% yield.

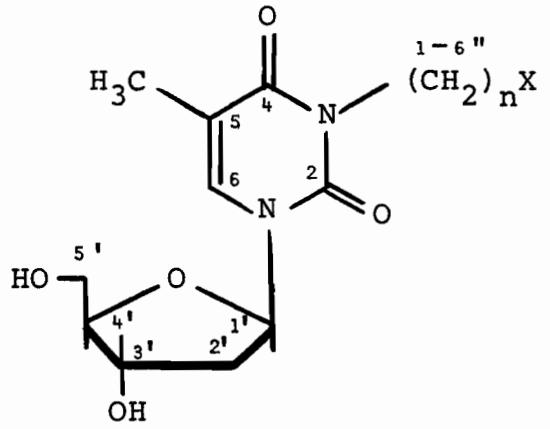


CXXIIIa, n=2, R=mmt, X=Cl
b, n=2, R=H, X=Cl
c, n=6, R=mmt, X=Cl
d, n=6, R=H, X=Br

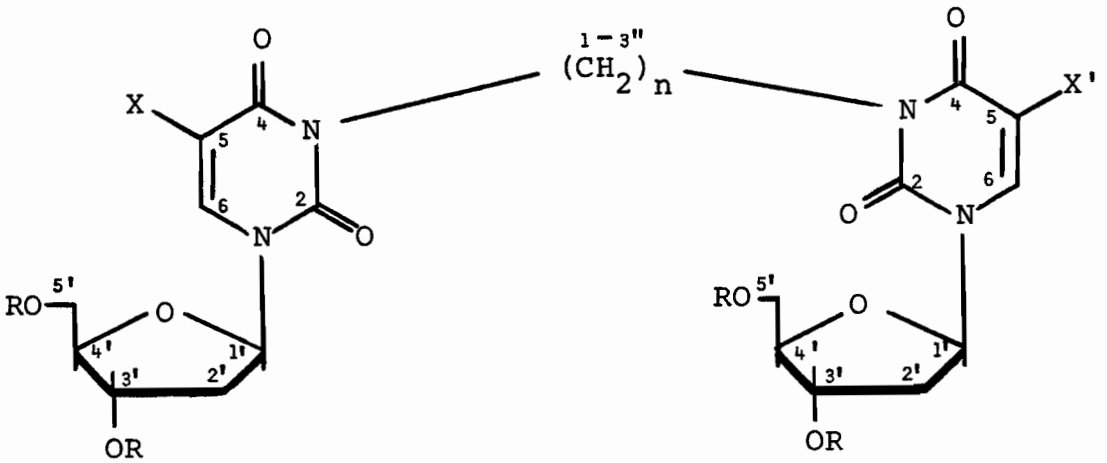


CXXIVa, n=2, R=mmt
b, n=2, R=H
c, n=6, R=mmt
d, n=6, R=H

CXXIIIb was characterized as for the previous cases by low and high resolution mass spectrometry. Its CMR spectrum indicates the presence of two extra peaks at 43.3 and 40.7 ppm with respect to thymidine. CXXIIId was prepared similarly by reacting LXIV with 1,6-dibromohexane. The structure was assigned because the CMR spectrum



CXVII a, n=1, X=F
CXXIIIb, n=2, X=Cl
CXXIIId, n=6, X=Br



CXVIIIa, n=1, X=X'=Me, R=H
CXXI , n=1, X=X'=H, R=H
CXXIa , n=1, X=X'=H, R=TBDMS
CXXIIa , n=1, X=Me, X'=H, R=H
CXXIIc , n=1, X=Me, X'=H, R=TBDMS
CXXIVb , n=2, X=X'=Me, R=H
CXXIVd , n=6, X=X'=Me, R=H

Table V. CMR Chemical Shift† Assignments for N^3-Haloalkyl and "Bridged" Nucleosides.

Compound	Solvent	C-2	C-4	C-5	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	Me	N^3-Alkyl Carbons C-1"	C-2"	C-3"	C-4"	C-5"	C-6"
T	DMSO d-6	150.5	163.8	109.5	136.2	83.9	39.5	70.5	87.3	61.4	12.3						
T	CD_3OD	--	166.1	111.6	138.1	86.3	41.2	72.2	88.8	62.8	12.4						
T^{CH_2F} (CXVIIa)	CD_3OD	151.7	164.2	110.7	137.8 137.4	87.1	41.0	71.9	88.7	62.6	12.9	84.5** 75.8**					
$T^{(CH_2)_2Cl}$ (CXXIIIb)	CD_3OD	152.5	165.3	110.6	136.9 136.6	87.1	41.3	72.0	88.8	62.7	13.1	43.3	40.7				
*$T^{(CH_2)_6Br}$ (CXXIIId)	CD_3OD	152.3	165.4	110.7	136.2	87.1	41.3	72.0	88.8	62.8	13.2	42.1	27.0	28.8	28.3	33.8	34.3
$T^{CH_2}T$ (CXVIIIa)	CD_3OD	151.6	164.6	110.4	136.7 136.4	86.8	41.1	71.8	88.5	62.6	13.0	40.4					
$T^{(CH_2)_2}T$ (CXXIVb)	CD_3OD	152.5	165.6	110.3	136.5	87.4	41.3	71.8	88.8	62.6	13.1	40.0					
*$T^{(CH_2)_6}T$ (CXXIVd)	CD_3OD	152.2	165.3	110.6	136.3	87.0	41.2	72.0	88.7	62.7	13.2	42.1	27.5	28.3			
U	DMSO d-6	151.2	163.8	102.2	141.6	88.3	74.0	70.3	85.2	61.3							
$dU^{CH_2}dU$ (CXXI)	CD_3OD	151.7	164.2	101.9	140.9	88.9	41.3	71.9	87.3	62.6		--					
††CXXIa	$CDCl_3$	150.3	162.1	101.6	138.3	87.7	41.9	71.2	85.8	62.4		46.3					
$T^{CH_2}dU$ (CXXIIa)	CD_3OD	151.8	164.7 164.3	110.6 (T) 101.9 (dU)	140.9 (dU) 136.7 (T)	88.9 (dU) 87.0 (T)	41.4 41.2	71.9	88.8 (T) 87.4 (dU)	62.7	12.9	--					
††CXXIIc	$CDCl_3$	150.3	162.9 162.1	109.9 (T) 101.6 (dU)	138.2 (dU) 133.8 (T)	87.7 (dU) 85.5 (T)	41.9 41.5	71.1 72.3	85.7 (dU) 87.8 (T)	62.4 63.1	13.2	46.8					

†δ in ppm with respect to TMS.

††Added for comparison.

*Tentative assignment for the N^3-alkyl carbons.

**$^1J_{C-F}$=197 Hz.

showed the presence of six extra peaks with respect to thymidine (Table V).

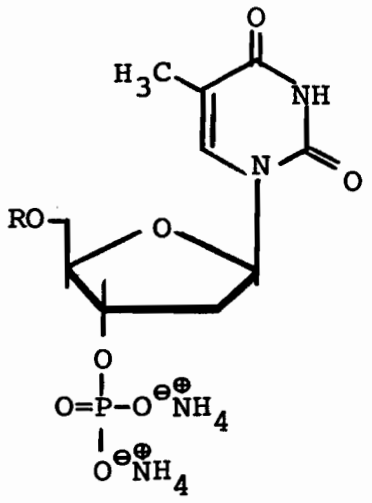
CXXIVa was also prepared from the reaction of CXXIIIa with thymidine (five molar equivalents) in the presence of TBAF (ten molar equivalents) in a concentrated DMF solution. After 62 hr, CXXIVa was isolated after work-up and purification on TLC plates in 27% yield. Because of symmetry, the CMR spectrum of CXXIVb exhibits one extra peak with respect to thymidine. On the other hand, low and high resolution mass spectrometry suggest the dimeric structure of CXXIVb.

CXXIVc was similarly prepared in THF instead of DMF. After a 15 hr reaction and usual work-up and purification procedures, CXXIVc was isolated in 35% yield. Again, for symmetry reasons the CMR spectrum of CXXIVd shows three extra peaks with respect to thymidine (Table V). As usual, low and high resolution mass spectrometry confirm the dimeric structure of CXXIVd. It is important to note the simplicity of the purification step. The excess thymidine and excess TBAF are removed by simple chloroform-water extractions. The organic phase contains only starting material and "bridged" product which are easily separated on TLC plates.

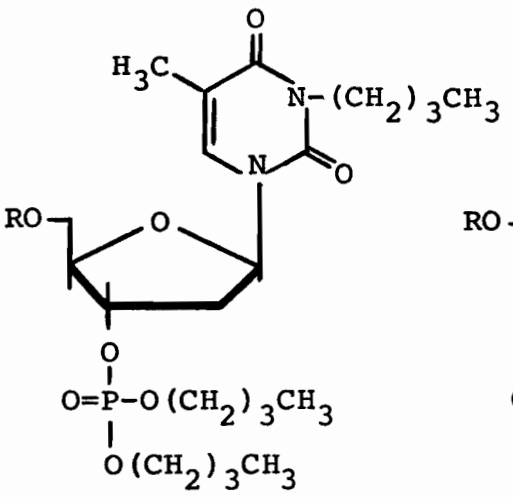
3. Alkylation of Nucleotides

For alkylation of nucleic acid derivatives, alkyl halides[210-214], dialkylsulfates[210,215-218], alkyl alkane-

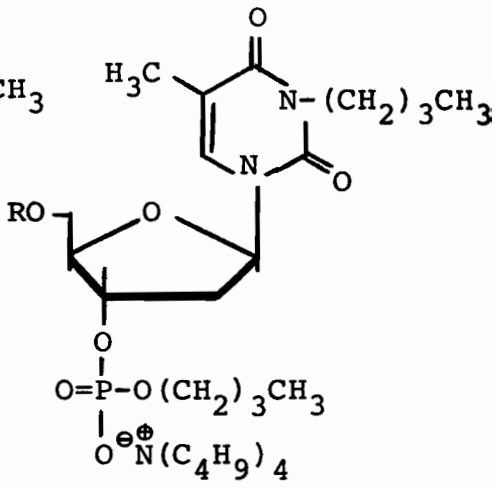
sulfonates[210,211,217,218], trialkylphosphates[219-221] and dialkyl phosphites[225] are generally employed as alkylating agents. Except for trimethylphosphate, the alkylating agents described above usually give yields under 60% employing temperatures as high as 220°C in some cases. Regardless of the fact that N,N-dimethylformamide acetals were found to be good alkylating agents for nucleic acid bases[226-230], the use of TBAF in the presence of alkyl halides, trialkyl phosphates, dialkylsulfates and alkyl alkanesulfonates, causes rapid and often quantitative alkylation of purine and pyrimidine bases at room temperature[231-232]. Furthermore, the mononucleotide and dinucleotide of thymidine, under these conditions and especially with dimethyl and diethyl sulfate give high yields of simultaneous triester formation and base alkylation[232]. However, with alkyl halide as alkylating agent, the yield of triester formation is moderate although base alkylation proceeds in nearly quantitative yield. For instance, the diammonium salt of 5'-O-trityl thymidine-3'-phosphate (CXXVa) was stirred with dry 1-chlorobutane in the presence of TBAF (ten molar equivalents) at 85°C for 24 hr. After work-up and purification on TLC plates, the triester CXXVIa ($trT^{Bu}p^{Bu}_{Bu}$) was isolated in 50% yield along with the diester CXXVIIa in 43% yield. When the same reaction was carried out at room temperature, CXXVIa and CXXVIIa were obtained in 20% and 67% yield respectively. The presence of the trityl group at the 5'-position



CXXV a, R=tr
b, R=H



CXXVIa,b



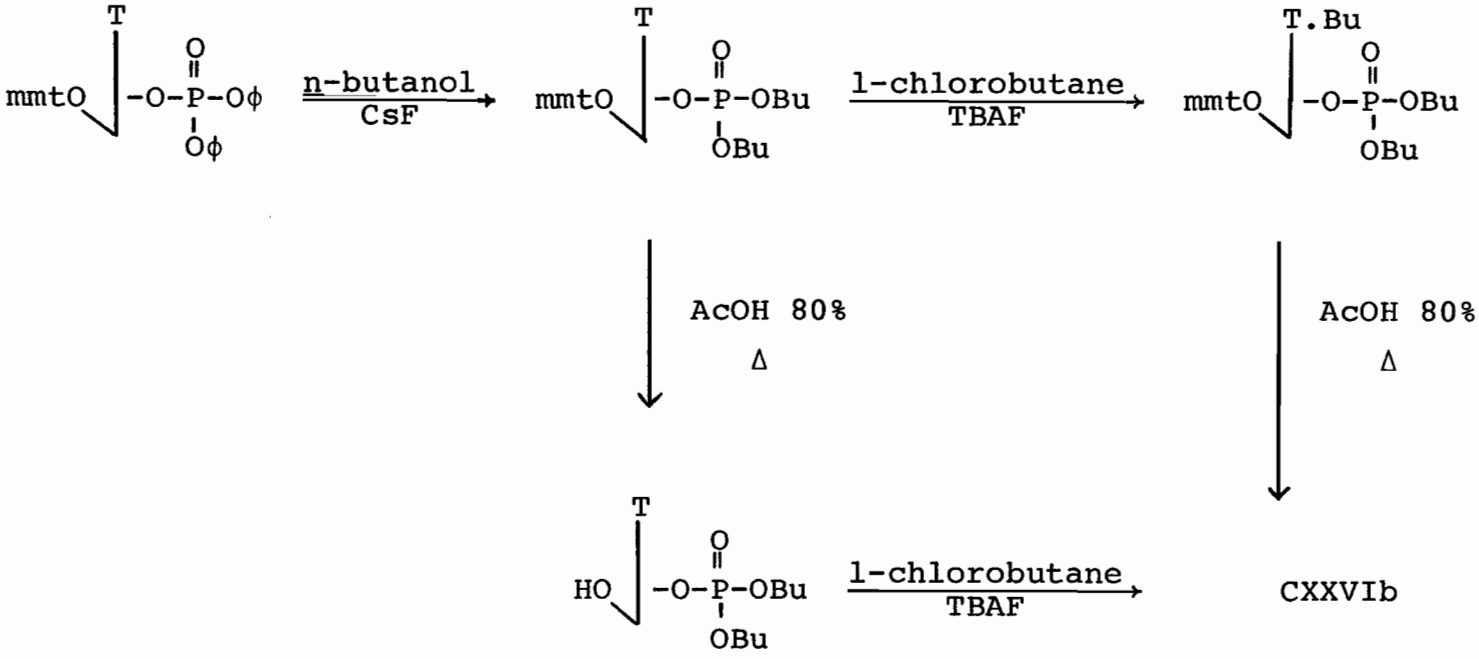
CXXVIIa,b

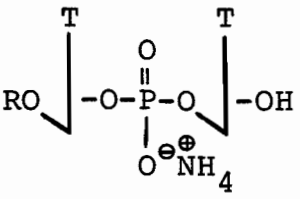
greatly facilitates work-up procedures. The concurrent triester formation and base alkylation occurred as well with the 5'-unprotected CXXVb as starting material with no trace of alkylation at the 5'-hydroxy function being observed. The structure of CXXVIb was checked by the synthesis of the compound using another route (Scheme XXIV).

The nature of the halide is important in the triester formation. Indeed, when 1-bromobutane is used as alkylating agent, CXXVIa was isolated in 40% yield compared to 20% when 1-chlorobutane was used. However, the triester formation seemed to stop when CXXVIa reached 50% yield even at 55-60°C with 1-bromobutane as alkylating agent.

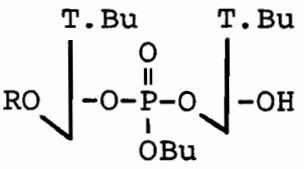
The reaction of the dinucleotide CXXVIIIa with 1-bromobutane in the presence of TBAF (ten molar equivalents) at 55-60°C for 24 hr gave the triester CXXIXa in 30% yield along

Scheme XXIV

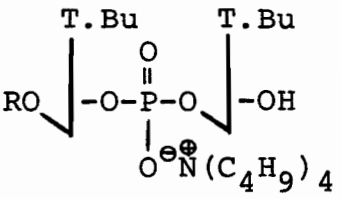

mmtO
T
O
P
Oϕ
n-butanol
CsF
OBu
1-chlorobutane
TBAF
T.Bu
AcOH 80%
Δ
HO
CXXVIb



CXXVIII a, R=mmt
b, R=H



CXXIXa,b



CXXXa,b

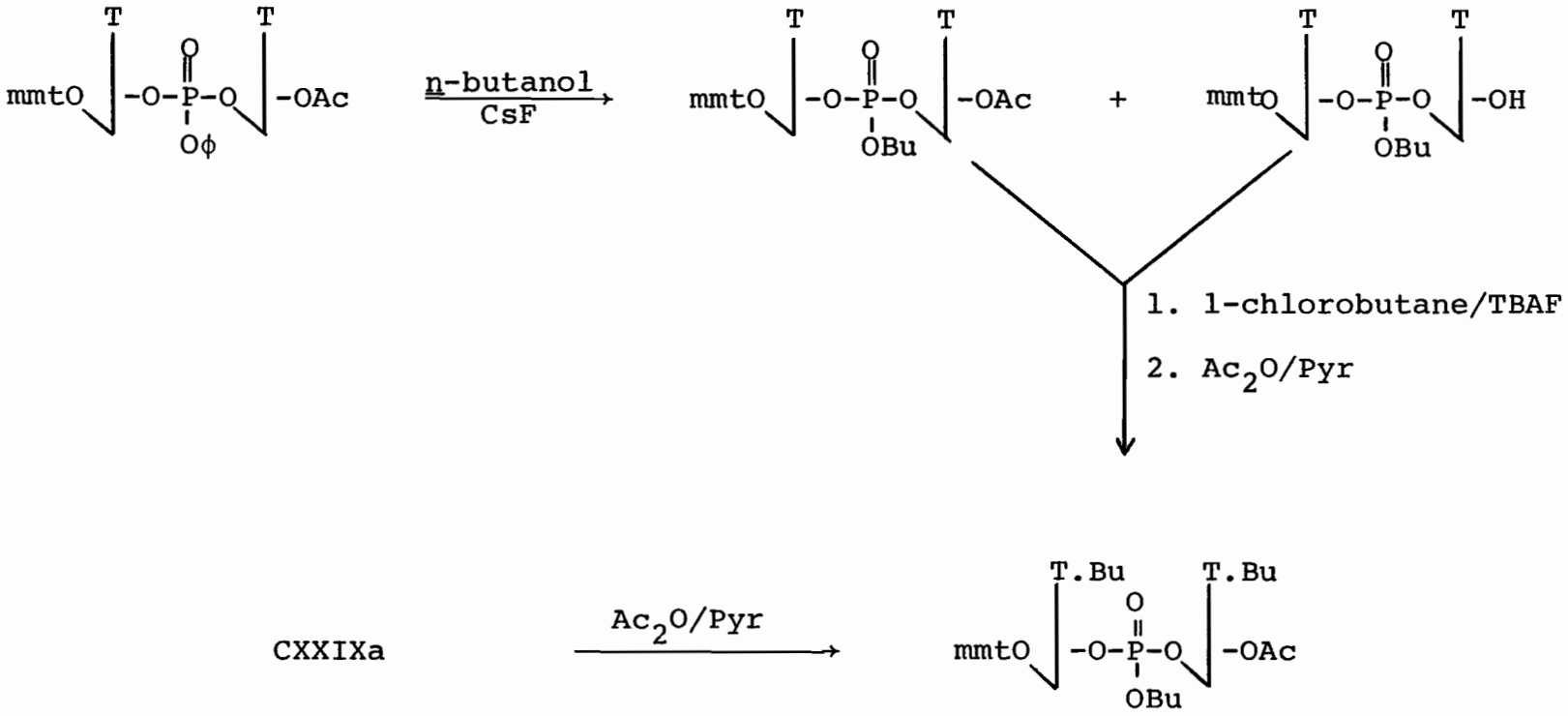
with the diester CXXXa in 63% yield.

The structure of CXXIXa was verified by its chemical synthesis via transesterification and alkylation procedures (Scheme XXV). Both CXXVIIb and CXXXb moved as singly charged compounds on paper electrophoresis. After purification on paper chromatography, the sodium salt of CXXXb was subjected to enzyme degradations. Bovine spleen phosphodiesterase degraded completely the substrate into $T^{Bu}p$ and T^{Bu} while snake venom phosphodiesterase degraded it completely to T^{Bu} and pT^{Bu}. These degradations showed that both bases were quantitatively alkylated.

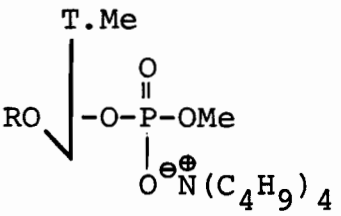
In an attempt to increase triester formation during the fluoride assisted alkylation of mono- and dinucleotides, other common alkylating agents including trialkylphosphates, dialkylsulfates and alkyl alkanesulfonates were investigated.

Treatment of CXXVa with trimethylphosphate in the presence of TBAF (ten molar equivalents) at room temperature for 24 hr yielded 95.4% of the diester CXXXIa. In order to be sure of the

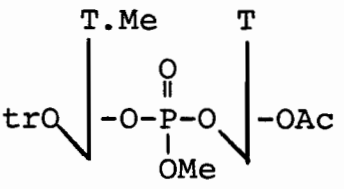
Scheme XXV


mmtO
-O-P-O
-OAc
Oϕ
n-butanol
CsF
OBu
+
-OH
1. 1-chlorobutane/TBAF
2. Ac2O/Pyr
CXXIXa
Ac2O/Pyr
T.Bu

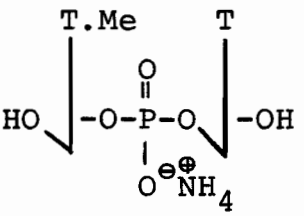
latter structure, the sodium salt of the diester was condensed with 3'-O-acetyl thymidine in the presence of TPS in pyridine, affording the fully protected dinucleotide CXXXII in 36% yield based on CXXXIa.



CXXXI a, R=tr
b, R=H



CXXXII

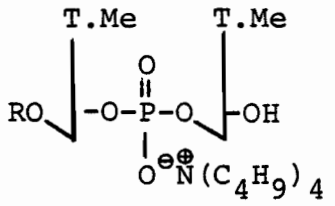


CXXXIII

The triester CXXXII was deprotected by treatment with concentrated ammonium hydroxide in pyridine and subsequently by 80% aqueous acetic acid. Preparative paper chromatography indicated that ca. 20% of the phosphotriester CXXXII was converted into the diester CXXXIII. After isolation, the latter was subjected to snake venom degradation. CXXXIII was completely degraded into T^{Me} and pT suggesting that the structure illustrated for CXXXIa is correct. It is important to mention here that TBAF is still required for the base alkylation and the triester formation. CXXVa was dissolved in trimethylphosphate by addition of a few drops of dry DMF and, in the absence of TBAF, the solution was stirred at room temperature for 67 hr. No triester formation and virtually no base alkylation (less

than 5%) were observed on TLC and on paper electrophoresis after work-up and deprotection of the reaction mixture with 80% aqueous acetic acid.

The reaction of the dinucleotide CXXVIIIa with trimethylphosphate and TBAF yielded after 24 hr at room temperature about 95% of the diester CXXXIVa, which was deprotected to CXXXIVb. After purification on paper chromatography, the sodium salt of CXXXIVb was submitted to phosphodiesterase degradations. Snake venom enzyme completely degraded the substrate into T^{Me} and pT^{Me} while the bovine spleen enzyme degraded it completely to $T^{Me}p$ and T^{Me}.



CXXXIV a, R=mmt
b, R=H

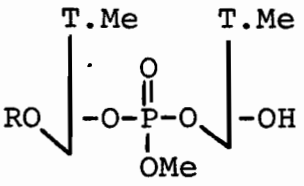
Trimethylphosphate did not promote triester formation in the presence of TBAF, but gave excellent yields of diester formation and base(s) alkylation for the synthesis of nucleotide analogs.

The use of dimethyl sulfate and diethylsulfate as alkylating agents in the presence of TBAF considerably improved triester formation from both mono- and dinucleotides. Most of

the alkylation reactions were carried out on a small scale using CXXVb and CXXVIIIb as starting materials. However, a few reactions were carried out on a larger scale using CXXVa and CXXVIIIa as starting materials. We wish to emphasize the work-up of these reactions. As soon as the reaction was finished (∿ 3 hr) the THF was removed under low pressure without heating. Caution must be taken to not evaporate the reaction mixture to dryness as this will result in an extensive detritylation of the reaction product giving rise to purification problems. Instead, the residue was poured into crushed ice mixed with a minimum of water. Sometimes the reaction product precipitated out nicely upon gentle manual stirring and sometimes an organic layer composed of the dialkylsulfate and the desired reaction product formed at the bottom of the beaker. In any case, the excess of TBAF and alkylsulfonic acid were removed by a careful filtration or decantation of the water. All other important details are given in the experimental section. We also observed on small scale reactions that five successive additions of five molar equivalents of TBAF and ten molar equivalents of the alkylating agent over a period of 3 hr often give better yields of reaction products compared to the reaction carried out with a simple addition of twenty-five molar equivalents of TBAF and fifty molar equivalents of the alkylating agent for the same period of time.

The fluoride assisted reaction of CXXVb and CXXVIIIb with

both dimethyl- and diethyl sulfate gave high yields of base alkylation[232] (virtually quantitative) and triester formation (82-98%, Table VI). These results are much better than those obtained in a reaction where methyl bromide was the alkylating agent where the triester CXXXVa was isolated in 55% yield along with CXXXIVa in 42% yield after 24 hr at room temperature.



CXXXV a, R=mmt
b, R=H

With methyl and ethyl methanesulfonate as alkylating agents, the base alkylation was still nearly quantitative but triester formation decreased drastically for both mononucleotides and dinucleotides (23-33%, see Table VI). All new triesters were identified by chemical syntheses , analogous to those described in Scheme XXI. Furthermore, $T^{Me}p_{Me}^{Me}$ and $T^{Et}p_{Et}^{Et}$ were characterized by mass spectral analysis which showed parent peaks at m/e = 364 and m/e = 406 respectively. All chromatographic properties of triesters and diesters as well as their electrophoretic and spectrophotometric properties are collected in Tables XIV and XV.

Because of the promising results obtained employing

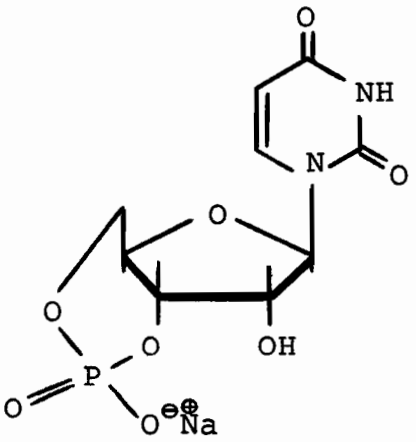
Table VI. Alkylation of Mononucleotide, Dinucleotide and Cyclic Phosphate with Dialkyl Sulfates and Alkyl Alkanesulfonates in the Presence of Fluoride Ions*

Starting Material	Alkylating Agent (molar eqiv.)	# of Additions	Time (hr)	O.D. units (Product, % yield)	
Tp(CXXVb)	$(MeO)_2SO_2$ (50)	5	3.5	76.3($T^{Me}p_{Me}^{Me}$, 95.6)	3.55($T^{Me}p_{OH}^{Me}$, 4.4)
Tp(CXXVb)	$(EtO)_2SO_2$ (50)	5	4.25	68.9($T^{Et}p_{Et}^{Et}$, 82.7)	14.5($T^{Et}p_{OH}^{Et}$, 17.3)
Tp(CXXVb)	$(MeO)SO_2Me$ (50)	5	3.25	12.7($T^{Me}p_{Me}^{Me}$, 31.1)	56.2($T^{Me}p_{OH}^{Me}$, 68.9)
Tp(CXXVb)	$(EtO)SO_2Me$ (50)	5	3.25	12.0($T^{Et}p_{Et}^{Et}$, 31.2)	53.0($T^{Et}p_{OH}^{ET}$, 68.8)
pU	$(MeO)_2SO_2$ (50)	5	3.0	97.9($_{Me}^{Me}pU^{Me}$, 94.6)	5.60($_{HO}^{Me}pU^{Me}$, 5.4)
pU	$(EtO)_2SO_2$ (50)	5	3.75	99.7($_{Et}^{Et}pU^{Et}$, 86.2)	15.9($_{HO}^{Et}pU^{Et}$, 13.7)
**(3'→5')cUMP(CXXXVI)	$(MeO)_2SO_2$ (25)	1	3.0	78.4($cU^{Me}p(Me)$, 83.8)	9.10($cU^{Me}p(OH)$, 9.7)
(3'→5')cUMP(CXXXVI)	$(EtO)_2SO_2$ (50)	5	4.25	75.8($cU^{Et}p(Et)$, 89.9)	8.50($cU^{Et}p(OH)$, 10.1)
(3'→5')cUMP(CXXXVI)	$(MeO)_2SO_2Me$ (50)	5	3.5	11.5($cU^{Me}p(Me)$, 15.4)	63.5($cU^{Me}p(OH)$, 84.6)
(3'→5')cUMP(CXXXVI)	$(EtO)SO_2Me$ (50)	5	3.5	15.4($cU^{Et}p(Et)$, 22.0)	54.6($cU^{Et}p(OH)$, 78.0)
TpT(CXXVIIIb)	$(MeO)_2SO_2$ (50)	5	3.5	155.0($T^{Me}p(Me)T^{Me}$, 97.8)	3.40($T^{Me}pT^{Me}$, 2.1)
TpT(CXXVIIIb)	$(EtO)_2SO_2$ (50)	5	3.0	119.9($T^{Et}p(Et)T^{Et}$, 83.8)	23.1($T^{Et}pT^{Et}$, 16.2)
TpT(CXXVIIIb)	$(MeO)SO_2Me$ (50)	5	3.0	15.3($T^{Me}p(Me)T^{Me}$, 23.1)	101.6($T^{Me}pT^{Me}$, 76.9)
TpT(CXXVIIIb)	$(EtO)SO_2Me$ (50)	5	3.5	23.7($T^{Et}p(Et)T^{Et}$, 32.7)	97.5($T^{Et}pT^{Et}$, 67.3)

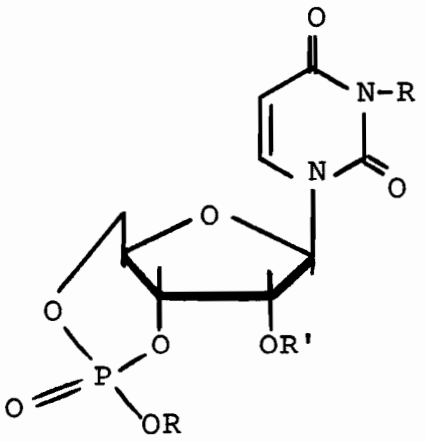
*15 molar equivalents of TBAF were used in this experiment.

**25 molar equivalents of TBAF were employed at room temperature unless otherwise indicated.

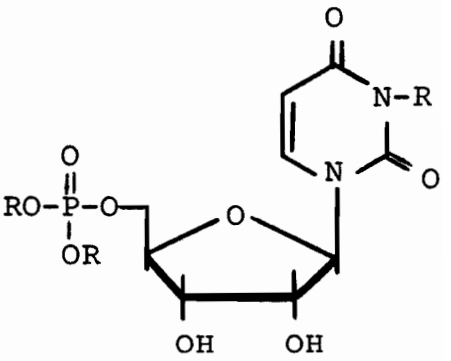
dimethyl- and diethyl sulfate as alkylating agents, it was decided to react the sodium salt of uridine-(3'→5') cyclic monophosphoric acid CXXXVI under similar conditions. In the presence of TBAF and dialkylsulfates, the triesters CXXXVIIa and CXXXVIIb were formed in 84% and 90% yield respectively. Alternatively, when alkyl alkanesulfonates were used as alkylating agents, CXXXVIIa and CXXXVIIb were respectively produced in only 15% and 22% yield, although base alkylation occurred in quantitative yield as in the previous cases. CXXXVIIIa,b were synthesized in high yields by the standard reaction with dialkylsulfates and TBAF (Table VI).



CXXXVI



CXXXVII a, R=Me, R'=H
b, R=Et, R'=H
c, R=Me, R'=TBDMS

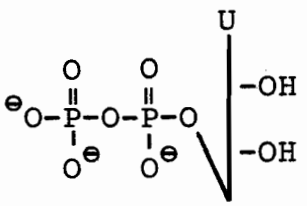


CXXXVIII a, R=Me
b, R=Et

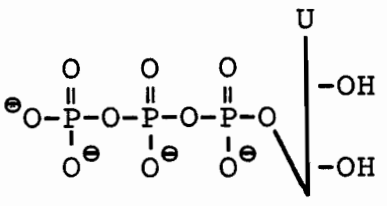
CXXXVIIa,b and CXXXVIIIa,b exhibit striking chromatographic differences on silica gel; the cyclic phosphates moving faster

than the corresponding noncyclic compounds. It was observed that the cyclic structure of CXXXVIIa,b was maintained during the course of the reaction because no trace of CXXXVIIIa,b was observed on TLC. The structure of CXXXVIIc was found to be correct by mass spectral analyses as indicated by the abundant $[M^{+}-57]$ ion due to loss of tert-butyl fragment at m/e = 391 (Fig. VIII).

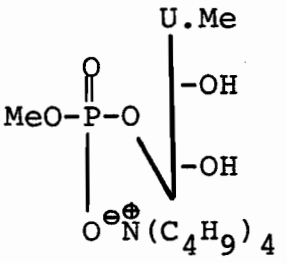
Interestingly, the pyridinium salts of uridine-5'-diphosphate (CXXXIX) and uridine-5'-triphosphate (CXL), when each was reacted with dimethylsulfate in the presence of TBAF under the usual conditions, gave after 3.25 hr the triester CXXXVIIIa in 36% and 23% yield respectively and the diester CXLI in 64% and 73% yield respectively.



CXXXIX



CXL



CXLI

CXLI was synthesized from CXXXVIIIa by treatment with TBAF (ten molar equivalents) in anhydrous THF for 60-90 min. All the diesters obtained from the alkylation reactions involving mono-, di- and triphosphate derivatives of uridine gave super-

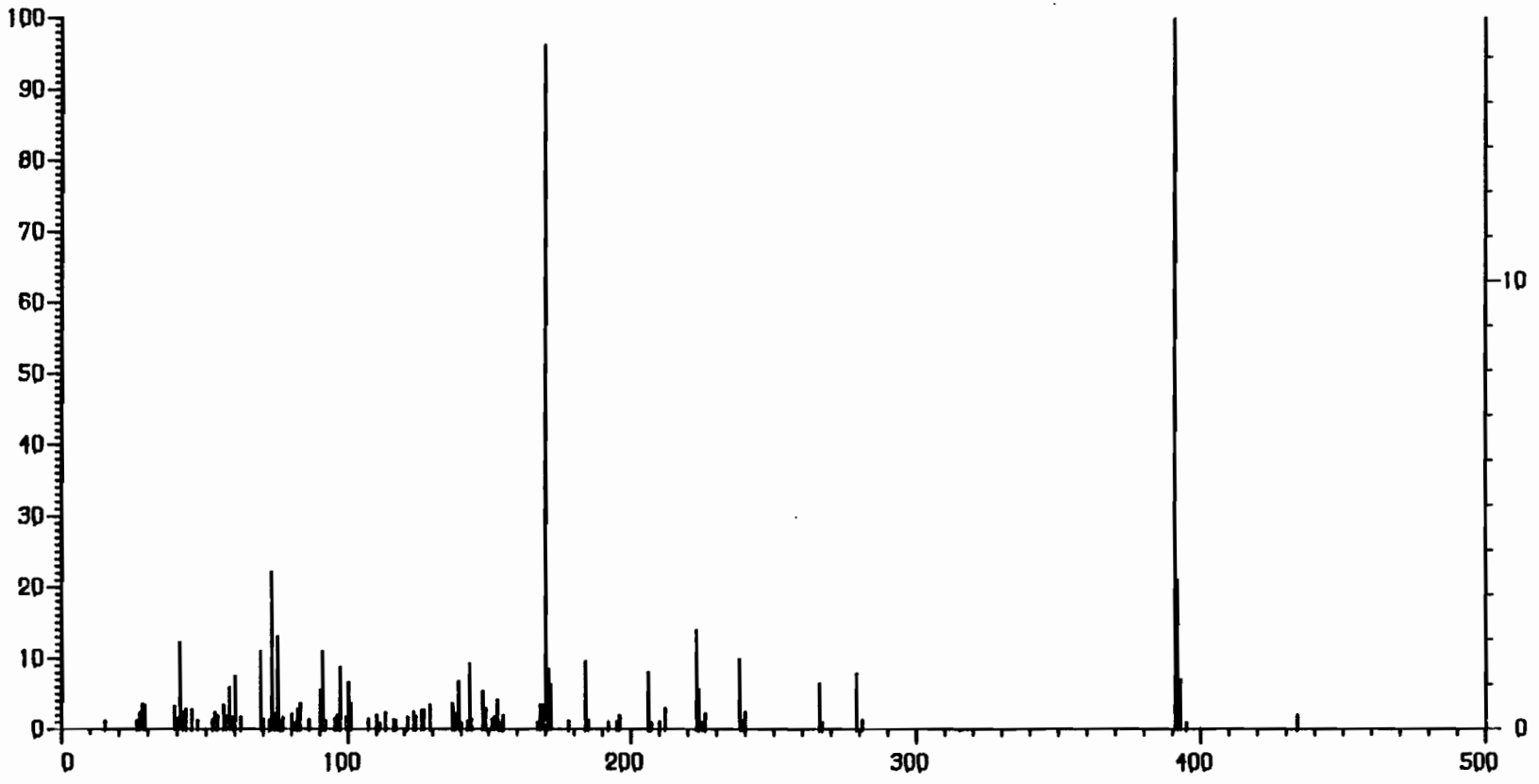


Fig. VIII. Mass Spectrum of the Methyl Ester of N^3-Methyl-2'-O-TBDMS Uridine-(3'→5') Cyclic Phosphate.

imposable PMR spectra as well as identical proton integrations. Furthermore, their paper chromatographic and electrophoretic properties proved to be identical (see Experimental section). Although these degradations are unexpected and interesting, the reaction mechanism is however not quite understood.

In conclusion, the alkylation of the phosphate function is relatively easy in the presence of tetrabutylammonium fluoride. This is demonstrated by the fact that many phosphate salts which are normally not soluble in THF dissolved readily upon addition of TBAF permitting better nucleophilic attack on the alkylating agent. Analogously, carboxylate ion is not considered a good nucleophile. However, when complexed with crown ethers, potassium salts of carboxylic acids become quite nucleophilic in non-polar solvents and enable very useful reactions such as alkylation[233], phenacyl ester and anhydride formation[234-236] as well as providing preparations of otherwise inaccessible lactone precursors[237]. It is then logical to believe that TBAF played some similar role in accounting for high yields of phosphotriester formation by certain alkylating agents.

CONTRIBUTIONS TO KNOWLEDGE

The tert-butyldimethylsilyl group proved to be stable under mild acidic conditions and phosphorylation conditions, thus permitting a stepwise synthesis of polynucleotide phosphotriesters. A selective fluoride assisted removal of the above silyl protecting group was achieved in the presence of fluoride sensitive phosphate protecting groups.

As subsequently pointed out by other workers, fluoride ion in either tetrahydrofuran or tetrahydrofuran-pyridine-water (8:1:1) was found to be unsuitable for the deprotection of dinucleotide phosphotriesters having phenyl or trichloroethyl phosphate protecting groups.

A simple and efficient synthesis of thymidine-3'-fluorophosphate was realized by the deprotection of a readily available mononucleotide triester by fluoride ion in both aprotic and protic solvents.

The fluoride promoted transesterification of triaryl and tris-trichloroethyl phosphates or thiophosphates led to the facile synthesis of certain cyclic phosphate triesters and analogs of biologically active phosphotriesters. The transesterification reaction was shown to be easily applicable to nucleotide phosphotriesters allowing the rapid introduction of a variety of alkyl groups at the phosphate. As an accomplishment, the synthesis of an analog of a DNA double helix

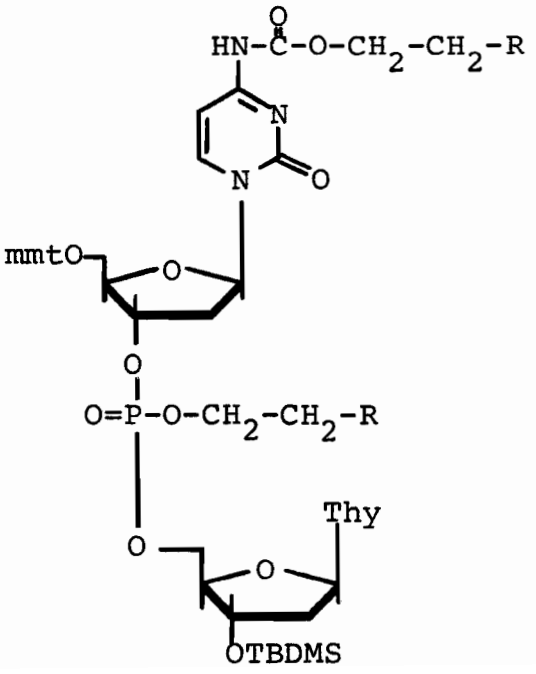
unit was achieved in two steps by this new procedure.

A new acylation method for nucleosides was developed using fluoride ion. The procedure allows the in situ replacement of a silyl group by an acyl protecting group without any side reaction at thymine and uracil bases. The reaction is generally fast, clean and efficient even for the acylation of sterically hindered hydroxyl groups.

Finally an unexpected fluoride induced alkylation reaction led to the synthesis of nucleoside dimers bridged through their thymine and/or uracil bases at the N^3-centres. With mono- and dinucleotides, this procedure gave, depending upon the alkylating agent used, a variety of phosphodiester and triester analogs as well as an interesting degradation of tri- and diphosphate derivatives of uridine.

ASPECTS FOR FURTHER INVESTIGATION

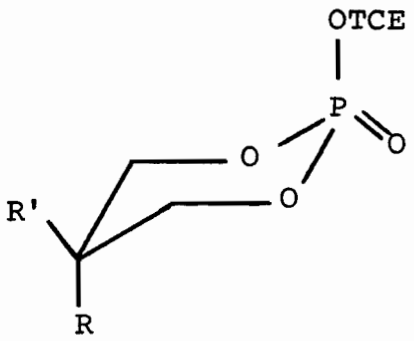
The deprotection of mmtTp(CE)TSi with fluoride ion in tetrahydrofuran-pyridine-water (8:1:1) (Reagent II) proceeds quantitatively via a β-elimination. Similarly the efficient deprotection of mmtTp(ϕ)TSi by CsF in 2-trimethylsilylethanol suggests the use of the 2-trimethylsilylethyl group as a phosphate protecting group. Its use in carboxyl protection has been demonstrated in peptide chemistry[239] and in the synthesis of natural compounds[240]. Moreover, the 2-trimethylsilylethoxycarbonyl group has been employed in the protection of the free amino-group of nucleosides bearing purine and pyrimidine bases[241]. Although the urethane that was formed showed some sensitivity to acidic conditions[241], it might be possible to replace the trimethylsilyl unit by the more acid stable tert-butyldimethylsilyl (TBDMS) or triisopropylsilyl (TIPS) unit and realize the synthesis of the following dinucleotide triester:



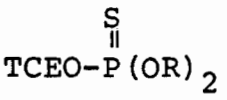
R = TBDMS or TIPS

It might be then possible to remove in one step all the silyl protecting groups of the above dinucleotide via a β-elimination in nearly quantitative yield using fluoride ion (Reagent II). Furthermore, fluoride ion immobilized on a polymer[242] in tetrahydrofuran-pyridine-water (8:1:1) might be tried as a deblocking agent, simplifying subsequent purification procedures.

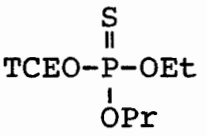
The facile synthesis of the cyclic phosphate XC from tris-trichloroethyl phosphate and 2,2-dimethyl-1,3-propanediol should stimulate the use of a variety of alcohols such as 1,3-propanediol or 2-methyl-1,3-propanediol in reactions with CsF to examine the possible cyclic phosphate formation of XCb,c. The synthesis of mixed trialkyl thiophosphates also deserve attention. Since Ca and Cb were synthesized in 83.3% and 81.4%, the synthesis of the thiophosphate CXLII by the reaction of XCIXa with n-propanol in the presence of CsF (ten molar equivalents) at 85°C for 38 hr should be achieved in good yield.



XCa, R=R'=Me
b, R=H, R'=H
c, R=H, R'=Me



Ca, R=Et
b, R=Pr

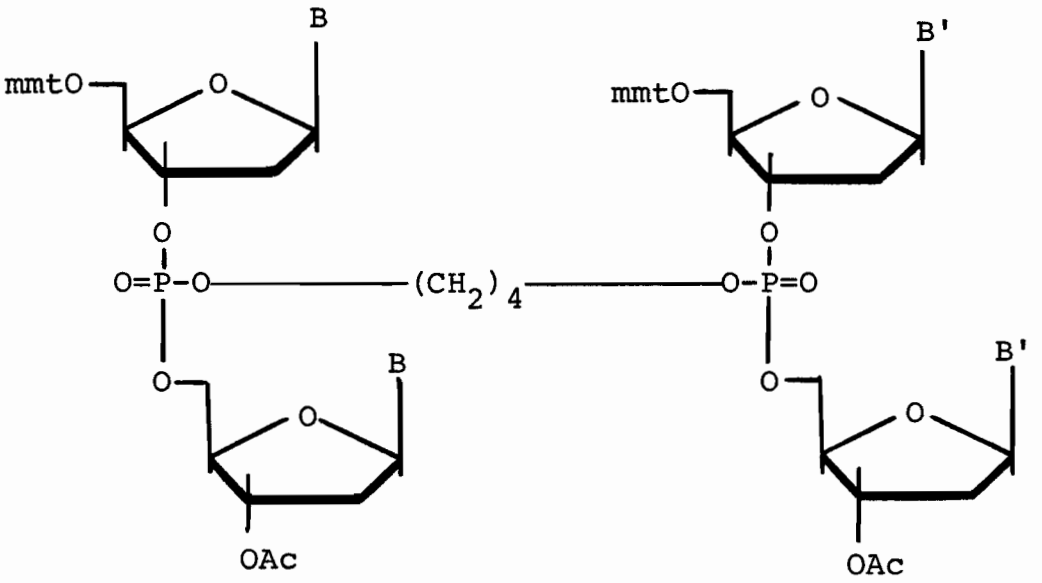


CXLII

Special attention should be given to the fluoride assisted syntheses of the thiophosphate analogs of XCa-c.

In addition to alcohols, amines should be investigated in the fluoride promoted transesterification reactions.

The synthesis of a symmetrical analog of a double stranded DNA unit (CVIIIa) was performed. The synthesis of an asymmetrical analog such as CXLIIIa or CXLIIIb is the logical extension of the procedure.



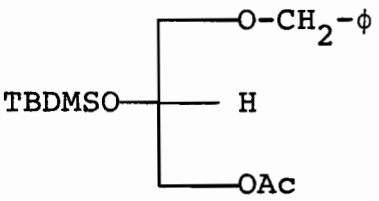
CVIIIa, B=B'=thymine

CXLIIIa, B=thymine, B'=N-benzoyladenine

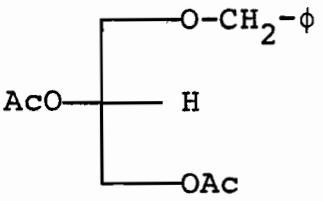
b, B=N-benzoylcytosine, B'=N-benzoylguanine

The fluoride assisted acylation reaction was successful with nucleosides and should find application in carbohydrate and synthetic chemistry in general.

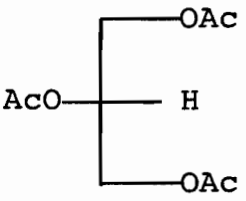
Furthermore, the fluoride induced silyl-to-acyl transformation might be an excellent complement of the ferric chloride in acetic anhydride procedure[198]. For instance, compound CXLIV would react with fluoride ion in acetic anhydride to afford the diacetate CXLV leaving the benzyl ether function untouched. On the other hand, with ferric chloride as catalyst, the triacetate CXLVI would be obtained.



CXLIV



CXLV



CXLVI

The alkylation procedure for nucleoside bases effected by fluoride should be promising for the alkylation of interesting organic compounds such as indole, β-carboline, fluorene and succinimide analogs. In addition, the facile synthesis of (3'→5') cyclic phosphate triesters of uridine from this procedure should promote investigations concerning the synthesis of (2'→3') cyclic phosphate triester analogs.

Finally, the detailed mechanism of the interesting degradation of tri- and diphosphate derivatives of uridine upon alkylation conditions should be scrutinized.

EXPERIMENTAL

General Methods and Equipment

Thin layer chromatography (TLC) was carried out employing the ascending technique in closed jars which were not coated with absorbent paper. All TLC's were run on Brinkman silica gel strips (6.7 cm x 2 cm) with fluorescent indicator. Thick layer chromatography was done on glass plates (20 cm x 20 cm) coated with a 1 mm thick layer of silica gel DSF-5 (Camag) purchased from Terrochem Laboratories.

Paper chromatography was performed by the descending technique using Whatman 3 MM paper sheets (55 cm x 23 cm). The solvent systems used were: Solvent A, isopropyl alcohol-concentrated ammonium hydroxide-water (7:1:2); Solvent B', n-butanol-ethanol-water (4:1:5, organic phase) and solvent F, n-propanol-concentrated ammonium hydroxide-water (55:10:35). The solvents were prepared on a volume basis. Products isolated from papers were generally obtained as solids by freeze-drying. For this purpose, a Virtis 10-010 automatic freeze-dryer was employed. Paper electrophoresis was performed using Whatman 3 MM paper (65 cm x 15 cm) in a Savant Flat Plate electrophoretic chamber with a Savant Model HV power supply operated at 2000 volts for 1.5 hr. The buffers used were

i) Phosphate buffer pH 8.0, prepared by dissolving 0.0033

mole of potassium dihydrogen phosphate and 0.063 mole disodium hydrogen phosphate in 1 litre of water.

ii) Triethylammonium bicarbonate buffer, 0.05 M, pH 7.5, prepared by adding 15.15 g of triethylamine to 3 litres of water, followed by slow addition of 20 g of dry ice and adjustment of the pH to 7.5 by adding dilute triethylamine or solid dry ice.

Electrophoretic mobilities (E_m) were calculated based on an E_m of 1.0 for thymidine-3'-phosphate. Nucleosides and nucleotides were visualized on TLC's and papers by using an ultraviolet light source (UVS-11, Mineralight output 254 nm). Compounds containing a trityl group or one of its derivatives were detected on chromatography support, by spraying with a 10% perchloric acid solution.

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Cary 17 spectrophotometer. G.l.c. analysis was performed with a Hewlett-Packard Model 5711A gas chromatograph equipped with a 10 ft x 1/8 in o.d. stainless steel column packed with 10% OV-1 on chromosorb W-HP.

Low resolution mass spectra were obtained from both magnetic sector LKB 9000 and Quadrupole Hewlett-Packard Model 59A4 mass spectrometers. High resolution mass spectra were obtained from an EIR MS 902 mass spectrometer. Molecular weight determination was performed by Galbraith Laboratories

Inc., Knoxville, Tennessee.

PMR spectra were recorded on a Varian T60-A NMR spectrometer. CMR spectra were obtained from a Bruker WH-90 FT NMR spectrometer.

Reagents and Chemicals

All nucleosides and certain mononucleotides were purchased from Sigma Chemical Co. tert-Butyldimethylsilyl chloride (TBDMSCl) and imidazole were purchased from Aldrich. TBDMSCl was stored in the refrigerator. Before use, it was allowed to warm up to room temperature to avoid the condensation of any moisture.

Reagent grade N,N-dimethylformamide (DMF), acetonitrile (MeCN) and 2,4,6-trimethylpyridine (Collidine) were refluxed over purified calcium hydride (Fisher) and then distilled at atmospheric pressure into reagent bottles containing molecular sieves "Linde" type 4A purchased from Matheson, Coleman and Bell. Reagent grade pyridine was kept overnight over p-toluenesulphonyl chloride and then refluxed for a minimum of 2 hr. It was distilled using a Vigreux column into a flask containing calcium hydride. Pyridine was subsequently distilled, using the Vigreux column, into reagent bottles containing molecular sieves (Type 4A).

β-cyanoethyl phosphate pyridinium salt was prepared from its barium salt (Sigma Chemical Co.) which was dissolved by

prolonged stirring in water. The slightly cloudy solution was slowly passed through a column filled with a DOWEX 50W-X8 ion-exchange resin (pyridinium form). The column was then washed with an equal volume of pyridine-water (1:9). All the washings were combined and then concentrated to a small volume under low pressure. The syrup was dissolved with water and lyophilized (∿ 24 hr). The thick oil was dissolved with dry pyridine until a 1 M solution was obtained. The amount of resin used was based on its specifications. The pyridinium form of the DOWEX 50W-X8 resin was prepared from its $H^{\oplus}$ form which was washed sequentially through a column with the following solutions: 0.1 M HCl until the washings were colorless; water until neutrality; pyridine-water (1:9) until basic; and finally with water to neutrality. The resin was then stored in water.

The preparation of the pyridinium salt of phenylphosphate was done according to the latter procedure.

Triisopropylbenzesulfonyl chloride (TPS) was obtained from Aldrich and was recrystallized from pentane prior to use.

Diphenylchlorophosphate and bis-(2,2,2-trichloroethyl) phosphorochloridate were obtained from Aldrich.

2,2,2-trichloroethylphosphorodichloridite[243] and phenylphosphorodichloridite[244] were prepared from phosphorus trichloride and the necessary alcohol as described in the literature.

Tetrahydrofuran (THF) was refluxed with sodium and benzophenone under nitrogen until the mixture turned to a deep purple color (2-3 hr). THF was then distilled under nitrogen into reagent bottles containing molecular sieves (Type 4A) and stored well protected from light.

Tetra-n-butylammonium fluoride (TBAF) was prepared by titration of a dilute (∿ 10%) aqueous solution of tetra-n-butylammonium hydroxide (obtained from Aldrich as a 32% aqueous solution) with a dilute (∿ 5%) aqueous solution of hydrofluoric acid (obtained from Alfa as a 49% aqueous solution) to pH 7.0 at 25°C. The neutral solution was then lyophilized (∿ 24 hr). The colorless and hygroscopic solid was reduced to a powder by shaking the flask, and dried further without heating over P_2O_5 for up to 24 hr. The dry powder was then dissolved with freshly distilled THF and stored over molecular sieves (Type 4A) in a plastic bottle tightly stoppered with a serum cap. Manipulation of the TBAF solution (0.68 M) was done with syringes. Alternate procedures for the preparation of anhydrous TBAF have also been published[245-248]. Recently, a 1 M solution of TBAF has become available from Aldrich[249]. Spectrograde methanol and reagent grade n-butanol, tert-butanol, ethylene glycol, 1,4-butanediol, 2-chloroethanol were dried according to standard procedures[250]. Absolute ethanol, iso-propanol, n-propanol, n-pentanol, 2-cyanoethanol and 2,2,2-trichloroethanol were used without any further drying.

2-trimethylsilylethanol* has been prepared according to the literature[251,252].

Triphenyl phosphate was purchased from Anachemia. Tris-trichloroethyl phosphate was synthesized by using a standard procedure[243]. bis-Trichloroethyl phenylphosphonate was similarly prepared from phenylphosphonic dichloride (Aldrich) and 2,2,2-trichloroethanol. Triphenyl thiophosphate and tris-trichloroethyl thiophosphate were prepared from the desulfurization of mercaptans by their corresponding triaryl and trialkyl phosphites[253,254]. 5,5-dimethyl-2-oxo-2-phenoxy-1,3,2-dioxaphosphorinane was obtained from the reaction of phenyldichlorophosphate (Aldrich) with 2,2-dimethyl-1,3-propanediol (Aldrich) under conditions described in the literature[255].

Anhdyrous potassium and cesium fluorides (KF and CsF) were obtained from Alfa.

Acetic anhydride was distilled prior to use. Trimethyl acetic anhydride and benzoic anhydride were obtained from Aldrich while myristic anhydride was commercially available from K & K Laboratories. No attempts were made to purify these anhydrides.

3',5'-di-O-TBDMS thymidine was prepared according to Ogilvie's procedure[173]. A pure sample of 2',5'-di-O-trityl uridine was provided by Dr. K.K. Ogilvie.

Anhydrous ferric chloride was purchased from Fisher and

*Donated by A.L. Schifman of our laboratory.

was used without further purification.

1-Chlorobutane, 1-bromobutane, dichloromethane and 1,2-dichloroethane were purified and dried according to standard procedures[250]. 1,6-dichlorohexane and 1,6-dibromohexane were acquired from Aldrich and were used directly. Methyl bromide (Eastman), methyl- and ethyl methanesulfonate (Eastman), trimethylphosphate (MCB), dimethylsulfate (J.T. Baker) and diethyl sulfate (Fisher) were employed without purification.

Enzyme Assays

i) Snake venom phosphodiesterase (Russel's Viper Venom). Two hundred units of the enzyme obtained from Calbiochem were dissolved with 1 ml of tris-(hydroxymethyl)aminomethane buffer (adjusted to pH 9.2 with 0.1 N HCl). About 0.1 ml of the enzyme solution was added to the nucleotide (0.1 to 1 mg) and incubated at 37°C for 7 hr. The solution was then applied to Whatman 3 MM paper as a band (5 cm length). The paper was developed in Solvent A. Nucleoside and nucleotide bands were cut out, eluted with water and diluted to a volume of 10 ml. The ultraviolet absorbance of the bands are given in terms of O.D. units which refer to the extinction of the absorbing material in 1 ml of neutral solution in a quartz cell with a 1 cm path length.

ii) Spleen phosphodiesterase (Bovine, Type I). About 23 units of the enzyme were dissolved with 1 ml of 0.01 M

sodium pyrophosphate buffer (adjusted to pH 6.5 with phosphoric acid). 0.1 ml of the enzyme solution was added to 0.1-1 mg of the nucleotide substrate which was previously dissolved with 0.2 ml of 0.05 M ammonium acetate (adjusted to pH 6.5 with acetic acid). The final solution was incubated at 37°C for 5 hr. The degradation products were separated as described for the snake venom case. Both enzymatic degradations constitute an absolute proof of structure for the substrate if the latter is degraded in a proper ratio.

Synthetic Methods

The tert-butyldimethylsilyl group as a protecting group in deoxynucleotide synthesis.

3'-O-TBDMS Deoxythymidine

5.44 g of 5'-O-p,p'-dimethoxytrityl thymidine (dmtT) (10 mmole) and 3.00 g of imidazole (44 mmole) were dissolved in 20 ml of distilled DMF. 3.32 g of TBDMSCl (22 mmole) was added and the solution was stirred at room temperature for 15 hr. TLC showed that the silylation was quantitative. The solution was then poured into 600 ml of ice water. The suspension was filtered on a coarse glass sintered funnel. The gummy solid was dissolved in acetone and passed through the funnel. A dry foam was obtained after evaporation of the solvent under reduced pressure. The white foam was then

dissolved with the minimum amount of 80% aqueous acetic acid (∿ 45 ml). The fully protected nucleoside was dissolved with gentle heating and the solution was then stirred at room temperature for 2-2.5 hr, the time required for a complete detritylation as checked by TLC. Acetic acid was removed under low pressure (T < 40°C) until a foam was obtained. The material was dissolved with the minimum of acetone and the solution was applied to a dry silica gel column (7 cm x 9 cm). The column was eluted with hexanes and ether-hexanes (1:1) in order to remove the p-p'-dimethoxytritanol and traces of unhydrolyzed dmtTSi. The desired compound was eluted from the column with ether. After removal of the solvent under reduced pressure, the residue was dissolved with the minimum of ether and was precipitated by addition of hexanes. Yield: 3.1 g (8.7 mmole , 87%).

N-benzoyl-3'-O-TBDMS Deoxycytidine

5.58 g of N-benzoyl-5'-O-p,p'-dimethoxytrityl deoxycytidine[172] (8.81 mmole) and 2.64 g of imidazole (38.8 mmole) were dissolved in 20 ml of distilled DMF. 2.92 g of TBDMSCl (19.4 mmole) was added and the solution was stirred at room temperature for 15 hr. The reaction mixture was then worked-up as in the previous preparation. The purification of the desired product was done by dry column chromatography using the same solvent system as for the previous preparation. Yield: 3.25 g

(7.29 mmole , 83%).

β-Cyanoethyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(CE)TSi)

130 mg of mmtT (0.25 mmole) and 0.5 ml of the pyridinium salt of β-cyanoethyl phosphate (βCEP, 0.5 mmole) were dried by coevaporation with dry pyridine (4 x 2 ml). 304 mg of TPS (1.0 mmole) was added and the mixture was dissolved in about 1.5 ml of dry pyridine. The solution was stirred at room temperature for 8 hr. TLC indicated that all mmtT had reacted. 1 ml of cold water was added and the solution was stirred at ambient temperature for 1-2 hr. The solution was then concentrated under low pressure to a small volume. 10 ml of chloroform ($CHCl_3$) was added and the resulting solution was extracted with water (4 x 3 ml). The organic layer was dried over anhydrous sodium sulfate (Na_2SO_4). The solvent was removed under low pressure, until a dry gum was obtained. 179 mg of 3'-O-TBDMS thymidine (0.5 mmole) was added to the gummy residue and the mixture was dried by coevaporation with dry pyridine as described above. 153 mg of TPS (0.5 mmole) was added and the mixture was dissolved in 1.5-2 ml of dry pyridine. The solution was stirred at room temperature for 24 hr. 1 ml of cold water was added and the solution was stirred for a further 1-2 hr. The reaction mixture was then submitted to similar work-up as for the first step described

above. The desired material was purified on silica gel plates which were developed with ether (Et_2O) and then with ethyl acetate (EtOAc). The dinucleotide triester was eluted from silica gel with freshly distilled THF. The solution was concentrated to a small volume (1-2 ml) under reduced pressure. 50 ml of hexanes was added and the desired triester precipitated as a white fluffy powder. Yield: 127 mg (0.13 mmole, 52%); m.p. 109-112°C; Rf: 0.07 (Et_2O), 0.37 (EtOAc); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 244 nm. The dinucleotide triester was completely deprotected into TpT. Chromatographic, electrophoretic and spectral properties of TpT are given in Table VII.

β-Cyanoethyl Phosphate Ester of Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (Tp(CE)TSi)

153 mg of mmtTp(CE)TSi (0.15 mmole) was dissolved with the minimum amount of 80% aqueous acetic acid. The solution was stirred at room temperature for 3 hr. Excess acetic acid was removed under low pressure and the syrup was dissolved with acetone and applied on TLC silica gel plates which were developed with Et_2O, EtOAc and finally with EtOAc-THF (1:1). The untritylated nucleotidic material was precipitated by addition of hexanes. Yield: 100 mg (0.14 mmole, 90%); m.p. 98-101 °C; Rf: 0.33 (EtOAc-THF (1:1)), 0.69 (THF); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 233 nm. Tp(CE)TSi was

employed in a trinucleotide triester synthesis without any further characterization.

β-Cyanoethyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N-benzoyl-3'-O-TBDMS Deoxycytidine (mmtTp-(CE)$dC^{Bz}Si$)

1.26 g of mmtT (2.45 mmole) and 4.9 ml of βCEP (4.9 mmole) were dried according to the standard procedure. 2.97 g of TPS (9.8 mmole) was added and the mixture was dissolved in dry pyridine (10 ml). After 8 hr of stirring at room temperature, the reaction was stopped by addition of water (4 ml). The solution was stirred for 2 hr and was then worked-up proportionately as usual. 1.2 g of N-benzoyl-3'-O-TBDMS deoxycytidine (2.70 mmole) was added to the diester and the mixture was dried by coevaporation with dry pyridine. 1.49 g of TPS (4.9 mmole) was then added followed by the minimum amount of dry pyridine (5 ml). The solution was stirred at room temperature for 24 hr and was then quenched by the addition of 2 ml of water. After 1 hr of stirring the reaction mixture was worked-up and purified on silica gel plates which were developed with Et_2O and then with EtOAc. MTrTp(CE)-$dC^{Bz}Si$ was isolated by precipitation from hexanes in 40% yield (1.05 g, 0.98 mmole); m.p. 117-122°C; Rf: 0.41 (EtOAc), 0.74 (EtOAc-THF (1:1)); u.v. (95% EtOH) λ_{max} = 262 nm, λ_{min} = 244 nm. The titled phosphotriester was completely deprotected into

TpdC which chromatographic, electrophoretic and spectral properties are given in Table VII.

β-Cyanoethyl Phosphate Ester of N-Benzoyl-5'-O-p-Monomethoxytrityl Deoxycytidilyl-(3'→5')-3'-O-TBDMS Thymidine ($mmtdC^{Bz}p(CE)TSi$)

This preparation was done under similar conditions and proportions as for the previous synthesis, but on a smaller scale. 300 mg of N-benzoyl-5'-O-p-monomethoxytrityl deoxycytidine (0.5 mmole) and 196 mg of 3'-O-TBDMS thymidine (0.55 mmole) were reacted. Purification was done on silica gel plates which were developed with Et_2O and then with EtOAc.

The nucleotidic material was isolated by precipitation with hexanes in 41% yield (218 mg, 0.20 mmole). After usual detritylation, the 5'-unblocked dinucleotide triester [m.p. 109-114°C; Rf: 0.05 (EtOAc), 0.54 (EtOAc-THF(1:1)); u.v. (95% EtOH) λ_{max} = 261 nm, λ_{min} = 230 nm] was further deprotected into dCpT for which chromatographic, electrophoretic and spectral data are reported in Table VII.

bis-β-Cyanoethyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(CE)Tp(CE)TSi)

115 mg of mmtT (0.22 mmole) and 0.45 ml of βCEP (0.45 mmole) were dried in the usual manner. 273 mg of TPS (0.90 mmole) was added and the mixture was dissolved in 2 ml of dry pyridine.

After 15 hr of stirring at room temperature, the reaction mixture was hydrolyzed for 2 hr by addition of 2 ml of water. After standard work-up, 80 mg of Tp(CE)TSi (0.11 mmole) was added and the mixture was dried by the usual coevaporation with dry pyridine. 133 mg of TPS (0.44 mmole) was added and the dry mixture was dissolved in the minimum of dry pyridine (2 ml). The solution was stirred at room temperature for 35 hr. The reaction was then quenched by addition of water and submitted to standard extraction procedure. Purification of the trinucleotide triester was achieved on TLC silica gel plates which were developed with Et_2O, EtOAc and EtOAc-THF (1:1). The triester was obtained in 50% yield after precipitation from hexanes (74 mg, 0.055 mmole); m.p. 145-155°C; Rf: 0.03 (EtOAc), 0.42 (EtOAc-THF (1:1)); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 244 nm. The nucleotidic triester was fully deprotected into TpTpT. Chromatographic, electrophoretic and spectral properties of the diester are listed in Table VII.

bis-Cyanoethyl Phosphate Ester of N-Benzoyl-5'-O-p-Monomethoxytrityl Deoxycytidilyl-(3'→5')-Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtdC^{Bz}p(CE)Tp(CE)TSi)

338 mg of N-benzoyl-5'-O-p-monomethoxytrityl deoxycytidine (0.56 mmole) and 1.15 ml of βCEP (1.15 mmole) were dried by the standard procedure. 679 mg of TPS (2.24 mmole) was added and the mixture was dissolved in 2 ml of dry pyridine. After

8 hr the reaction was stopped by addition of 2 ml of water. Standard work-up was then performed. To the dry mixture composed of 375 mg of Tp(CE)TSi (0.53 mmole) and 0.56 mmole of the diester was added 340 mg of TPS (1.12 mmole) followed by the minimum amount of dry pyridine (2 ml). After 24 hr the reaction was stopped by the addition of 2 ml of water. After 2 hr the reaction mixture was worked-up as usual. Purification on TLC silica gel plates was effected by successive developments with Et_2O, EtOAc and EtOAc-THF (1:1). The triester was obtained in 42% yield after addition of hexanes (320 mg, 0.22 mmole). The trinucleotide triester was fully deblocked into dCpTpT. Table VII illustrates the chromatographic, electrophoretic and spectral data of the diester. dCpTpT was quantitatively degraded by both snake venom and bovine spleen phosphodiesterases. The ratios of their degradation products are given in Table VIII.

bis-Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidine ($mmtTp^{\phi}_{\phi}$)

200 mg of mmtT (0.39 mmole) was dried by coevaporation of dry pyridine (4 x 2 ml). 210 mg of diphenylchlorophosphate (0.16 ml, 0.78 mmole) was added to the nucleoside previously dissolved in 2 ml of dry pyridine. The solution was then stirred at room temperature for 15 hr. 1 ml of water was

then added and the solution was stirred for a further 15-30 min. Solvent was removed under low pressure until a gum was obtained. The material was then taken up in $CHCl_3$ (20 ml) and then extracted with water (4 x 6 ml). The organic layer was concentrated to a small volume under reduced pressure. The product was purified on TLC silica gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O. The product was eluted from silica gel with acetone. The solvent was then removed under low pressure leaving a white solid. Yield: 264 mg (0.35 mmole, 90%); m.p. 76-77°C; Rf: 0.18 (Et_2O-hexanes (3:1)), 0.28 (Et_2O); u.v. (95% EtOH) λ_{max} = 266, 263 nm, λ_{min} = 248 nm.

The yield is reproducible on large scale by using dry silica gel column chromatography and the same solvent system for the elution.

bis-(2,2,2-Trichloroethyl) Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidine ($mmtTp^{TCE}_{TCE}$)

514 mg of mmtT (1 mmole) was dried as usual and then dissolved in 5 ml of dry pyridine. 758 mg of bis-(2,2,2-trichloroethyl) phosphorochloridate (2 mmole) was added and the solution was stirred at room temperature for 24 hr. Water (2 ml) was added and after 30 min the reaction mixture was worked-up and purified on TLC silica gel plates using identical solvent systems as for the previous preparation. The mononucleotide

triester was isolated in 90% yield (770 mg, 0.90 mmole); m.p. 92-93°C; Rf: 0.23 (Et_2O-hexanes (3:1)), 0.34 (Et_2O); u.v. (95% EtOH) λ_{max} = 266 nm, λ_{min} = 254 nm.

Stability of $mmtTp^{\phi}_{\phi}$ and $mmtTp^{TCE}_{TCE}$ Toward Ammonium Hydroxide (NH_4OH)

To 10 mg of $mmtTp^{\phi}_{\phi}$ and 10 mg of $mmtTp^{TCE}_{TCE}$ in two separate screw cap vials was added 2 ml of concentrated ammonium hydroxide. The suspensions were dissolved by the dropwise addition of pyridine. The clear solutions were stirred at room temperature and the rates of hydrolysis were checked by TLC. It required 1.5 hr for the complete hydrolysis of $mmtTp^{TCE}_{TCE}$ and 8 hr for $mmtTp^{\phi}_{\phi}$. One half of each reaction was then taken and evaporated to dryness under low pressure. The residues were dissolved with 80% aqueous acetic acid and the solutions were stirred at 80°C for 15-30 min. Excess acetic acid was removed under reduced pressure and the nucleotidic materials were spotted on electrophoresis paper and on preparative papers which were developed in solvent B'. For both reactions, electrophoresis showed a singly charged product and no traces of a doubly charged material suggesting that the diesters Tp^{ϕ}_{OH} and Tp^{TCE}_{OH} were exlusively formed during the alkaline hydrolysis. Paper chromatography for both reactions revealed two bands, the faster corresponding to thymidine. The bands were cut out and eluted with water and their

respective yields were obtained spectrophotometrically. The remaining half-reactions were worked-up similarly after 24 hr. Results are summarized below:

R	Time (hr)	% Tp^{R}_{OH}	% T
ϕ	8	96.4	3.6
ϕ	24	96.5	3.5
TCE	1.5	81.1	18.9
TCE	24	80.8	19.2

This procedure was applied successfully on large scale. Purification of the mononucleotide diesters was done by dry silica gel column chromatography. Elution was first carried out with Et_2O in order to remove phenol or 2,2,2-trichloroethanol and most of mmtT. Subsequent washing with ethyl acetate completed the removal of mmtT. The desired diester was eluted with $CHCl_3$-MeOH (7:3).

Stability of $mmtTp^{\phi}_{\phi}$ and $mmtTp^{TCE}_{TCE}$ Toward 80% Aqueous Acetic Acid

20 mg each of $mmtTp^{\phi}_{\phi}$ and $mmtTp^{TCE}_{TCE}$ was separately dissolved in 2 ml of 80% aqueous acetic acid. The solutions were heated at 80°C. The rate of hydrolysis was checked by electrophoresis after 15 min, 30 min, 1 hr and 2 hr. After 120 min at 80°C electrophoresis showed only traces of the diester Tp^{ϕ}_{OH} (<< 5%) as a singly charged compound (E_m = 0.52) while Tp^{TCE}_{TCE} appeared

to be untouched under these conditions.

Stability of mmtTp$^{\phi}_{OH}$ and mmtTp$^{TCE}_{OH}$ Toward 80% Aqueous Acetic Acid

20 mg of the ammonium salt of each diester was dissolved in 2 ml of 80% aqueous acetic acid in separate vials. The solutions were heated at 80°C for 2 hr. Meanwhile the reaction mixtures were spotted on paper electrophoresis after 15 min, 30 min, 60 min and 120 min. After 2 hr, only the presence of Tp$^{\phi}_{OH}$ (E_m = 0.52) and Tp$^{TCE}_{OH}$ (E_m = 0.55) was observed. There was no trace of the monoester Tp (E_m = 1.00) indicating that both diesters are stable under these conditions.

Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(ϕ)TSi)

150 mg of the ammonium salt of mmtTp$^{\phi}_{OH}$ (0.22 mmole) and 39 mg of 3'-O-TBDMS thymidine (0.11 mmole) were dried by coevaporation with dry pyridine (4 x 2 ml). 133 mg of TPS (0.44 mmole) was added and the mixture was dissolved in 2 ml of dry pyridine. The solution was stirred at room temperature for 36 hr. Water (1 ml) was added and the solution was stirred for a further 2 hr. Solvent was then removed under reduced pressure. The residue was taken up in $CHCl_3$ (10 ml) and then extracted with water (4 x 3 ml). The $CHCl_3$ layer was decanted, concentrated to a small volume and applied on silica

gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O. The desired compound was eluted from silica gel with methanol. The solution was evaporated to dryness under low pressure. The residue was dissolved with the minimum amount of dry THF (2 ml) and the dinucleotide triester was precipitated by the addition of hexanes (50 ml). Yield: 81 mg (0.08 mmole, 73%); m.p. 105-110°C; Rf: 0.25 (Et_2O), 0.63 (EtOAc); u.v. (95% EtOH) λ_{max} =265 nm, λ_{min} = 245 nm. The above triester was completely deprotected into TpT which showed identical properties as for those reported in Table VII.

Phenyl Phosphate Ester of Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (Tp(ϕ)TSi)

91 mg of mmtTp(ϕ)TSi (0.090 mmole) was dissolved in 15 ml of 80% aqueous acetic acid. The solution was stirred at room temperature for 3 hr. The solvent was then removed under low pressure and the residue was purified on plates which were consecutively developed with Et_2O and twice with EtOAc. After standard isolation procedure Tp(ϕ)TSi was obtained in 92% yield (61 mg, 0.083 mmole); m.p. 97-102 °C; Rf: 0.15 (EtOAc), 0.73 (EtOAc-THF (1:1)); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 233 nm. Tp(ϕ)TSi was utilized in a trinucleotide triester synthesis without further characterization.

2,2,2-Trichloroethyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(TCE)TSi)

100 mg of the ammonium salt of $mmtTp^{TCE}_{OH}$ (0.14 mmole) and 75 mg of 3'-O-TBDMS thymidine (0.21 mmole) were dried as usual. 85 mg of TPS (0.28 mmole) was added and the mixture was dissolved in 2 ml of dry pyridine. The solution was stirred for 36 hr. Work-up and purification on silica gel plates were identical as for the preparation of mmtTp(ϕ)TSi. Yield: 97 mg (0.09 mmole, 65%); m.p. 117-122°C; Rf: 0.31 (Et_2O), 0.63 (EtOAc); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 246 nm. As usual mmtTp(TCE)TSi was fully deblocked into TpT which showed identical properties as those reported in Table VII.

2,2,2-Trichloroethyl Phosphate Ester of Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (Tp(TCE)TSi)

The selective removal of the mmt group was realized on the same scale and under the same conditions as for the preparation of Tp(ϕ)TSi. A 92% yield of Tp(TCE)TSi (66 mg, 0.083 mmole) was isolated from silica gel plates. m.p. 105-110°C; Rf: 0.17 (EtOAc), 0.78 (EtOAc-THF (1:1)); u.v. (95% EtOH) λ_{max} = 266 nm, λ_{min} = 233 nm. Tp(TCE)TSi was used directly in the synthesis of a trinucleotide triester.

Phenyl Phosphate Derivative of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N-Benzoyl-3'-O-TBDMS Deoxycytidine (mmt-Tp(ϕ)dC^{Bz}Si)

1.3 g of the ammonium salt of $mmtTp^{\phi}_{OH}$ (1.94 mmole) and 446 mg of N-benzoyl-3'-O-TBDMS deoxycytidine (1 mmole) were dried following standard procedure. 1.18 g of TPS (3.88 mmole) was added and the mixture was dissolved in dry pyridine (5 ml). The solution was stirred at ambient temperature for 36 hr. After usual work-up, the product was purified on silica gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O. 509 mg of mmtTp(ϕ)dC^{Bz}Si (0.46 mmole, 46% yield) was isolated. m.p. 108-113°C; Rf: 0.29 (Et_2O-EtOAc(1:1)), 0.56 (EtOAc); u.v. (95% EtOH) λ_{max} = 261 nm, λ_{min} = 244 nm. The mmt group of the titled phosphotriester was selectively removed as usual and the resulting Tp(ϕ)dC^{Bz}Si was used without any characterization in the synthesis of mmtdC^{Bz}p(ϕ)Tp(ϕ)dC^{Bz}Si.

bis-Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(ϕ)Tp(ϕ)TSi)

545 mg of the ammonium salt of $mmtTp^{\phi}_{OH}$ (0.82 mmole) and 300 mg of Tp(ϕ)TSi (0.41 mmole) was dried as customary. 254 mg of TPS (0.84 mmole) was added and the mixture was dissolved in 2 ml of dry pyridine. The solution was stirred at room

temperature for 36 hr. After standard work-up, the product was purified on silica gel plates which were developed with Et_2O-hexanes (1:1), Et_2O and Et_2O-EtOAc (1:1). The trinucleotide triester was isolated in 69% yield as a white solid (389 mg, 0.28 mmole); m.p. 113-118°C, Rf: 0.29 (EtOAc), 0.73 (EtOAc-THF (1:1)); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 244 nm. The selective removal of the mmt group was conventionally accomplished. Tp(ϕ)Tp(ϕ)TSi was directly involved in the synthesis of mmtTp(ϕ)Tp(ϕ)Tp(ϕ)TSi without further characterization

bis-(2,2,2-Trichloroethyl) Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(TCE)Tp(TCE)TSi)

180 mg of the ammonium salt of $mmtTp^{TCE}_{OH}$ (0.24 mmole) and 98 mg of Tp(TCE)TSi (0.12 mmole) were dried. 145 mg of TPS (0.48 mmole) was added and the mixture was dissolved in 2 ml of dry pyridine. The solution was then stirred at ambient temperature for 36 hr. After usual work-up the reaction mixture was applied on silica gel plates which were developed with Et_2O-hexanes (1:1), Et_2O and finally with Et_2O-EtOAc (1:1). The conventional isolation procedure gave 129 mg of a white powdered trinucleotide triester (0.086 mmole, 72% yield; m.p. 116-121°C, Rf: 0.41 (EtOAc), 0.80 (EtOAc-THF (1:1)); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 244 nm. The triester was converted into TpTpT which exhibits identical properties as

those listed in Table VII.

bis-Phenyl Phosphate Ester of N-Benzoyl-5'-O-p-Monomethoxytrityl Deoxycytidilyl-(3'→5')-Thymidilyl-(3'→5')-N-Benzoyl-3'-O-TBDMS Deoxycytidine (mmtdC^{Bz}p(ϕ)Tp(ϕ)dC^{Bz}Si)

1.34 g of N-benzoyl-5'-O-p-monomethoxytrityl deoxycytidine (2.22 mmole) and 4.44 ml of a 1.0 M solution of the pyridinium salt of phenyl phosphate (4.44 mmole) were dried according to the usual manner. 2.69 g of TPS (8.88 mmole) was added and the mixture was dissolved in 5 ml of dry pyridine. After 15 hr at room temperature, TLC showed the presence of some starting material. 2.22 mmole of the pyridinium salt of phenyl phosphate was added along with TPS (4.44 mmole). The solution was then stirred at ambient temperature for a day. After habitual work-up, the diester and 700 mg of Tp(ϕ)dC^{Bz}Si (0.85 mmole) were dried. 1.34 g of TPS (4.44 mmole) was added and the mixture was dissolved in 5 ml of dry pyridine. The solution was allowed to stir at room temperature for 36 hr. After conventional work-up and purification on silica gel plates which were developed with Et_2O, and then with EtOAc, the desired phosphotriester was obtained in 24% yield (313 mg, 0.20 mmole). The selective removal of the mmt group from the fully protected trinucleotide was realized as customary. The resulting 5'-unblocked trinucleotide triester [m.p. 124-129°C; Rf: 0.52 (EtOAc-THF(1:1)), 0.80 (THF); u.v. (95% EtOH) λ_{max} =

260 nm, λ_{min} = 230 nm] was directly used in the synthesis of the tetranucleotide triester described below.

tris-Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N-Benzoyl-Deoxycytidilyl-(3'→5')-Thymidilyl-(3'→5')-N-Benzoyl-3'-O-TBDMS Deoxycytidine (mmtTp(ϕ)dC^{Bz}p(ϕ)Tp-(ϕ)dC^{Bz}Si)

182 mg of the ammonium salt of $mmtTp^{\phi}_{OH}$ (0.27 mmole) and 175 mg of $dC^{Bz}p(\phi)Tp(\phi)dC^{Bz}Si$ (0.135 mmole) were dried carefully. 164 mg of TPS (0.54 mmole) was added and the dry mixture was dissolved in 1 ml of dry pyridine. The solution was stirred at room temperature for 36 hr. After work-up, the reaction was purified on silica gel plates which were developed with Et_2O, EtOAc and EtOAc-THF (1:1). 97 mg of the fully protected tetranucleotide was isolated (0.05 mmole, 37%). m.p. 115-120°C, Rf: 0.10 (EtOAc), 0.80 (EtOAc-THF(1:1)); u.v. (95% EtOH) λ_{max} = 261 nm, λ_{min} = 243 nm. The tetranucleotide triester was fully deprotected into TpdCpTpdC which chromatographic, electrophoretic and spectral properties are given in Table VII. TpdCpTpdC was quantitatively degraded by both snake venom and bovine spleen phosphodiesterases. The ratios of their degradation products are given in Table VIII.

tris-Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidilyl-(3'→5')-Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTp(ϕ)Tp(ϕ)Tp(ϕ)TSi)

429 mg of the ammonium salt of $mmtTp^{\phi}_{OH}$ (0.745 mmole) and 416 mg of Tp(ϕ)Tp(ϕ)TSi (0.372 mmole) were conventionally dried. 451 mg of TPS (1.49 mmole) was added and the solid mixture was dissolved in 4 ml of dry pyridine.

The reaction mixture was stirred at ambient temperature for 36 hr. After the usual work-up, the mixture was purified on silica gel plates which were developed with Et_2O, Et_2O-EtOAc (1:1) and EtOAc. 290 mg of the fully protected tetranucleotide was obtained (0.164 mmole, 44% yield). m.p. 110-115°C; Rf: 0.31 (EtOAc), 0.80 (EtOAc-THF(1:1)) ; u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 243 nm. The above tetranucleotide triester was converted into TpTpTpT which exhibits the properties given in Table VII. Table VIII displayed the ratios of the degradation products obtained from the quantitative degradation of TpTpTpT by both snake venom and bovine spleen phosphodiesterases.

Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidine (mmtTp(ϕ)T)

38 mg of mmtTp(ϕ)TSi (0.037 mmole) and 1.09 g of phenylacetic acid (8 mmole) were dissolved in 1 ml of dry THF.

0.59 ml of a solution of 0.68 M TBAF (0.4 mmole) was added and the solution was stirred at room temperature for 15-20 hr. The solvent was then removed under low pressure and $CHCl_3$ (5 ml) was added to the residue. The resulting solution was extracted several times with a 10% aqueous sodium bicarbonate solution ($NaHCO_3$) (2 ml) until no phenylacetic acid was left in the $CHCl_3$ phase. The organic layer was then washed with water (2 ml) until the aqueous phase became neutral. The $CHCl_3$ layer was concentrated to a small volume under reduced pressure and then applied on silica gel plates which were developed with Et_2O and twice with EtOAc. After standard isolation procedure, 29 mg of pure mmtTp(ϕ)T was obtained (0.033 mmole, 89% yield). m.p. 103-108°C; Rf: 0.56 (EtOAc-THF (1:1)), 0.71 (THF); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 245 nm.

Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N-Benzoyl Deoxycytidine (mmtTp(ϕ)dC^{Bz})

1.0 g of mmtTp(ϕ)dC^{Bz}Si (0.92 mmole) and 25.0 g of phenylacetic acid (184 mmole) were dissolved in 100 ml of dry THF. 13.5 ml of a solution of 0.68 M TBAF (9.2 mmole) was added and the solution was stirred at ambient temperature for 24 hr. The reaction mixture was then proportionately worked-up as for the previous preparation. Purification of the reaction product was done on silica gel plates which were developed with Et_2O and then with EtOAc-THF (1:1). 855 mg of mmtTp(ϕ)dC^{Bz}

was obtained (0.87 mmole, 95% yield); m.p. 116-121°C; Rf: 0.41 (EtOAc), 0.79 (EtOAc-THF(1:1)) ; u.v. (95% EtOH) λ_{max} = 261 nm, λ_{min} = 244 nm.

2,2,2-Trichloroethyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidine (mmtTp(TCE)T)

35 mg of mmtTp(TCE)TSi (0.033 mmole) and 872 mg of phenylacetic acid (6.4 mmole) were dissolved in 1 ml of dry THF. 0.47 ml of a solution of TBAF (0.33 mmole) was added and the solution was stirred at room temperature for 15-20 hr. The reaction mixture was then submitted to identical work-up and purification procedures as for the previous preparations. 28 mg of mmtTp(TCE)T was isolated (0.030 mmole, 90% yield); m.p. 119-124°C; Rf: 0.58 (EtOAc-THF (1:1)), 0.75 (THF); u.v. (95% EtOH) λ_{max} = 266 nm, λ_{min} = 246 nm.

Stability of the Trichloroethyl Phosphate Protecting Group Toward the Selective Removal of the 3'-O-TBDMS Protecting Group

In order to check the stability of the trichloroethyl phosphate protecting group, the following experiment was carried out: 5 mg of mmtTp(TCE)TSi (0.005 mmole) was dissolved in 1 ml of dry THF. 0.12 ml of glacial acetic acid (2 mmole) was added followed by 0.075 ml of a solution of 0.68 M TBAF (0.051 mmole). The reaction mixture was stirred at room

temperature for 24 hr and was then left without stirring for 5 days. After a total time of 6 days the solution was evaporated under reduced pressure. The material left was dissolved with 80% aqueous acetic acid (4 ml) and the solution was heated at 80°C for 15 min. Excess acetic acid was removed under vacuum and the residue was applied on preparative papers which were developed in solvent A.

The two bands accounting for Tp(TCE)T and TpT were cut out and eluted with water and their respective yields were acquired spectrophotometrically. 64.0 $O.D._{266}$ units of Tp(TCE)T (87.6%) and 9.1 $O.D._{266}$ units of TPT (12.4%) were obtained.

Studies on the deprotection of mono- and dinucleotide triesters by fluorides in aprotic solvents.

General Procedure for the Deprotection of Mononucleotide Triesters by Reagent I and Reagent II

0.02 mmole of the mononucleotide triester was dissolved in Reagent I. (0.18 ml of a solution of 0.68 M TBAF (0.12 mmole) and 0.5 ml of dry THF) or in Reagent II (0.18 ml of a solution of TBAF 0.68 M (0.12 mmole) and 0.5 ml of a solution of THF:pyridine:water (8:1:1). With Reagent I, reaction mixtures were stirred at room temperature for 30 min. Reaction times were 24 hr with Reagent II. Reactions were then quenched by addition of water and evaporated under reduced

pressure. If a mononucleotide triester bearing 5'-O-mmt protecting group was used, this residue was dissolved in 80% aqueous acetic acid and the solution was stirred at 85°C for 30 min. Excess acetic acid was removed under low pressure and work-up continued as follows. The residue was dissolved in a solution of water-ethanol (1:1). The solution (2-4 ml) was applied to a column (1 cm x 35 cm) filled with an ion-exchange resin (DOWEX 50W-X8, $Na^{\oplus}$ ionic form). The column was eluted with water (30-35 ml) at the rate of ∿ 1 drop/sec. The aqueous solution was evaporated under low pressure and the nucleotidic material was purified on preparative papers which were developed with solvent B'. Bands were cut out and eluted with water and their respective yields were determined spectrophotometrically. Results are given in Tables I and VIIIa.

General Procedure for the Deprotection of Dinucleotide Triesters by Reagent I

0.01 mmole of dinucleotide triester was dissolved in Reagent I (0.15 ml of a solution of 0.68 M TBAF (0.1 mmole) and 0.5 ml of dry THF) or in Reagent II (0.15 ml of a solution of 0.68 M TBAF (0.1 mmole) and 0.5 ml of a solution of THF:pyridine:water (8:1:1). Reaction mixtures were stirred for 1 hr with Reagent I or 24 hr with Reagent II. After a work-up identical as for the deprotection of mononucleotide triesters, the reaction mixtures were purified on preparative

papers which were developed with solvent A. The yields of the bands were secured spectrophotometrically and the results are reported in Table I.

Paper Chromatographic, electrophoretic and spectral data of deprotected mono- and dinucleotides as well as degradation fragments are given in Table VIIIa.

General Procedure for the Deprotection of Polynucleotides Having Nucleoside Bases Bearing N-Protected Amino Groups

0.01 mmole of polynucleotide triester was dissolved in Reagent II (3-5 molar equivalents of TBAF per silyl and phosphate protecting group in 0.5-1.0 ml of a solution of THF: pyridine:water (8:1:1)). The solution was stirred at room temperature for 24 hr. The reaction mixture was then evaporated under low pressure. The nucleotidic material was dissolved in a solution of concentrated ammonium hydroxide in pyridine (1:1) (10 ml) and the solution was stirred at ambient temperature for 24 hr. The reaction mixture was then evaporated to dryness under reduced pressure and was treated with 80% aqueous acetic acid (10 ml) at 85°C for 30 min. After evaporation of acetic acid under vacuum, the nucleotidic material was worked-up and purified on preparative papers as for the previous deprotection reactions.

Table VII. Paper Chromatographic, Electrophoretic and Ultraviolet Properties of Fully Deprotected Polynucleotides.

Compound	B'	A	F	E_m*	$\lambda^{H_2O}_{max}$ (nm)	$\lambda^{H_2O}_{min}$ (nm)
TpT	0.05	0.43	0.68	0.44	266	234
TpdC	0.02	0.36	0.66	0.40	267	237
dCpT	0.02	0.32	0.65	0.41	268	240
dCpTpT	0.02	0.18	0.54	0.66	267	236
TpTpTpT	0.00	0.08	0.47	0.77	266	234
TpdCpTpdC	0.00	0.06	0.43	0.78	266	236

*Electrophoretic mobilities after 1.5 hr in triethylammonium bicarbonate buffer (0.05 M, pH = 7.5).

Table VIII. Enzymatic Degradation of Polynucleotides

Compound		Snake Venom Phosphodiesterase				Bovine Spleen Phosphodiesterase			
		T	pT	dC	pdC	T	Tp	dC	dCp
dCpTpT	Calcd.		2	1		1	1		1
	Found		1.93	1.00		1.00	0.93		1.01
TpdCpTpdC	Calcd.	1	1		2		2	1	1
	Found	1.00	0.94		2.02		1.94	1.00	1.03
TpTpTpT	Calcd.	1	3			1	3		
	Found	1.00	2.95			1.00	2.96		

Table VIIIa. Paper Chromatographic and Spectral Properties of Products Obtained from the Fluoride Assisted Deprotection of Nucleotide Triesters.

Compound	B'	A	E_m*	$\lambda^{EtOH\ 95\%}_{max}$ (nm)	$\lambda^{EtOH\ 95\%}_{min}$ (nm)
Tp^{F}_{OH}(LXXIII)	0.18	0.63	0.62	266	233
Tp^{ϕ}_{OH}(LXXIVa)	0.37	0.69	0.52	265	234
Tp^{TCE}_{OH}(LXXIVb)	0.48	0.72	0.54	267	236
Tp^{CE}_{OH}	0.12	0.57	0.57	266	236
cTMP(LXXV)	0.07	0.51	0.68	265	234
TpT(LXXVII)	0.05	0.43	0.44	266	234
X†	0.05	0.39	0.44	266	234
TpTp(ϕ)T(LXXXIb)	--	0.50	0.25	266	236
TpTpT	0.02	0.16	0.62	266	233

*Electrophoretic mobilities after 1.5 hr in triethylammonium bicarbonate buffer (0.05 M, pH = 7.5).

†Product obtained from the deprotection of Tp(ϕ)TSi and Tp(TCE)TSi with Reagent I.

Deprotection of the 2,2,2-Trichloroethyl Phosphate Ester of 5'-O-p-Monomethoxytrityl-2'-O-TBDMS Uridilyl-(3'→5')-2',3'-di-O-TBDMS Uridine (LXXVIII)

13 mg of the titled phosphotriester (0.01 mmole) was dissolved with Reagent II (0.22 ml of a solution of 0.68 M TBAF (0.15 mmole) in 0.5 ml of a solution of THF:pyridine:water (8:1:1)) and the solution was stirred at room temperature for 24 hr. The reaction mixture was then directly passed through the ion-exchange resin column. After evaporation of the aqueous solution under low pressure, the tritylated nucleotide was purified on preparative papers which were developed in solvent A. Bands were cut out, eluted with water and those corresponding to mmtUp(TCE)U and mmtU were eluted with 95% EtOH. Their yields were determined spectrophotometrically. Results are listed on Table I.

Phenyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-Thymidilyl-(3'→5')-3'-O-TBDMS Thymidine (mmtTpTp(ϕ)TSi, LXXXIa)

53 mg of $mmtTp^{\phi}_{\phi}$ (0.071 mmole) was dissolved in 5 ml of dry THF. 1.04 ml of a solution of 0.68 M TBAF (0.71 mmole) was added and the solution was stirred at room temperature for 1 hr. The solvent was removed under low pressure and the residue was dissolved in $CHCl_3$ (10 ml) which was then extracted with

water (4 x 3 ml). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The remaining gum was washed several times with anhydrous ether (4 x 20 ml) in order to eliminate phenol. The gum became a slightly yellow solid. 26 mg of Tp(ϕ)TSi (0.035 mmole) was added and the mixture was carefully dried by coevaporation with dry pyridine. TPS (43 mg, 0.142 mmole) was added along with 1 ml of dry pyridine. The solution was stirred at room temperature for 37 hr. After usual hydrolysis and extraction procedures, the nucleotidic material was dissolved in EtOH 95% up to a volume of 25 ml.

6.5 ml of the stock solution (∿ 0.01 mmole of mmtTpTp(ϕ)TSi) was evaporated to dryness under low pressure. The residue was dissolved in 80% aqueous acetic acid and the solution was stirred at 85°C for 5 hr. The solvent was then removed under vacuum and the nucleotidic material was purified on preparative papers which were developed in solvent A. The band corresponding to TpTp(ϕ)T (Rf^A = 0.48, E_m = 0.25) was eluted with water and then lyophilized. The light solid was treated with 0.14 ml of a solution of 0.68 M TBAF (∿ 0.1 mmole) and 0.5 ml of dry THF or dry pyridine. After 8 hr electrophoresis showed only starting material (E_m = 0.25). The solution was then evaporated to dryness under low pressure and the residue was dissolved in a solution of sodium hydroxide 0.2 N (1-2 ml). The solution was stirred at room temperature for 14 hr. The alkaline solution was neutralized with an ion-

exchange resin (DOWEX 50W-X8, pyridinium form) and evaporated under reduced pressure. Both paper chromatography and electrophoresis showed TpTpT as the major product (Rf^A = 0.17, E_m = 0.62). 6.5 ml of the stock solution was evaporated to dryness under vaccum. The nucleotidic material was dissolved in 9 ml of a solution of potassium hydroxide 0.1 N in H_2O-DMSO (1:9). The solution was stirred at room temperature for 3 hr. The solution was then neutralized by the addition of an ion-exchange resin (DOWEX 50W-X8, pyridinium form). After filtration, the aqueous solution was evaporated to dryness. The material was then dissolved in a solution of hydrochloric acid 0.2 N in dioxan-water (1:1) (10 ml) and heated at 80°C for 15 min. After evaporation of the solution under low pressure, the fully deprotected nucleotidic material was purified on preparative papers which were developed in solvent A. Bands corresponding to TpTpT and TpT were cut out and eluted with water and their respective yields were determined spectrophotometrically. 40.7 $O.D._{266}$ units of TpTpT (69%) and 18.5 $O.D._{266}$ units of TpT (31%) were observed. The yield of TpT was corrected by taking into consideration the extinction coefficient of the dinucleotide.

Transesterification reactions induced by fluoride ion on triaryl, trialkyl and nucleotide phosphotriesters.

General Procedure for the Fluoride Ion Promoted Transesterification in Non-Nucleotide Phosphate Triesters

0.135 mmole of the phosphate triesters ($(\phi O)_3P=O$, $(\phi O)_3P=S$ and $(TCEO)_3P=S$) and 0.675 mmole or 1.35 mmole of CsF were dissolved with 1 ml of the appropriate alcohol. The solutions were monitored by gas chromatography (g.l.c.) as follows: Aliquots (0.1 ml) of the reaction mixtures were added to 0.3 ml of ether and 0.5 ml of water in a stoppered vial which was rigorously shaken. After the separation of phases, injections for g.l.c. were taken from the organic phase. Peaks corresponding to products were cut from the chromatograms and their yields were calculated based on the papers' weights. Results for reactions giving some selectivity in the removal of phosphate protecting groups are given in Table IX.

Unusual Fluoride Assisted Transesterification of Triphenylphosphate with Hydracrylonitrile

To 100 mg of triphenyl phosphate (0.31 mmole) in 1 ml of hydracrylonitrile (2-cyanoethanol) was added 465 mg of CsF (3.1 mmole). A clear solution was obtained after heating with hot water. After 15-20 hr at ambient temperature an aliquot

Table IX. Transesterification of Non-Nucleotidic Phosphate Triesters Induced by Fluoride Ions.

$(RO)_3P{=}S$	R'OH	(molar equiv.) CsF	T(°C)	Time(hr)	$(RO)_3P{=}S$(%)	$(RO)_2\overset{S}{\overset{\|}{P}}\text{-}OR'$(%)	$(R'O)_2\overset{S}{\overset{\|}{P}}\text{-}OR$(%)	$(R'O)_3P{=}S$(%)
R=ϕ	R'=Et	5	25	72	32.9	55.7	11.4	--
"	"	"	"	144	10.1	63.4*	14.8	11.7
"	"	"	50	23	8.4	60.0*	16.2	15.4
"	"	"	"	39	0.0	38.1	27.6	34.2
"	"	10	"	23	0.0	16.2	45.4*	38.4
"	"	"	"	32	0.0	4.5	43.0	52.5
R=TCE	"	2	65	37	11.1	82.3	6.5	--
"	"	"	"	88	4.1	85.3*	10.7	--
"	"	5	70	17	5.6	85.5*	8.9	--
"	"	"	"	43	0.0	57.8	38.4	3.8
"	"	10	65	16	2.2	87.7*	10.0	--
"	R'=i-Pr	"	"	16	5.4	72.1	22.5	--
"	"	"	"	24	0.0	75.1*	24.9	--
"	R'=n-Pr	"	"	4	25.2	70.0	4.8	--
"	"	"	"	14	2.4	81.8*	13.7	2.1
"	R'=Et	"	85	22	0.0	15.1	77.7	7.2
"	"	"	"	38	0.0	0.0	83.3*	16.6
"	"	"	95	8	0.0	23.8	71.6	4.5
"	"	"	"	15	0.0	0.0	81.1*	18.9
"	"	"	"	23	0.0	0.0	77.0	23.0
"	R'=n-Pr	"	85	22	0.0	21.1	72.9	6.1
"	"	"	"	38	0.0	4.4	81.4*	14.1

*Optimum overall yields obtained.

was poured into a NMR tube along with some deuteromethanol (CD_3OD). The presence of acrylonitrile was revealed by peaks between 5.5-6.5 ppm in the PMR spectrum (see Fig. I). These peaks were identical to those in a spectrum of pure acrylonitrile.

Besides acrylonitrile, peaks in the PMR spectrum between 6.7-7.3 ppm indicated that the rest of the reaction mixture consisted of phenyl phosphate mono- and diesters and phenol.

The reaction of hydracrylonitrile (1 ml) with 465 mg of CsF gave after 20 hr at room temperature no detectable amount of acrylonitrile according to PMR.

Ethyl, (2,2,2-Trichloroethyl), Phenylphosphonate (LXXXIII)

1 g of bis-(2,2,2-trichloroethyl), phenylphosphonate (LXXXII, 2.38 mmole) and 1.8 g of CsF (11.88 mmole) were dissolved in absolute ethanol (17.4 ml). The solution was stirred at room temperature. The rate of the reaction was monitored by g.l.c. (Table X). After 22.5 hr, the solution was then submitted to the usual $CHCl_3$-water extractions. The reaction product was purified on preparative silica gel plates which were developed twice with ether-hexanes (1:1). LXXXIII was isolated as an oil in 51% yield (384 mg, 1.21 mmole).

PMR ($CDCl_3$, Fig. II): 7.2-8.0 ppm (5H, m, ϕ)

4.4-4.6 ppm (2H, d, P-O-$\underline{CH_2}$-CCl_3),

$^2J_{P-O-CH_2}$ = 7 Hz

3.8-4.4 ppm (2H, q, $-O-\underline{CH_2}-CH_3$)

1.2-1.4 ppm (3H, t, $-O-CH_2-\underline{CH_3}$)

iso-Propyl, (2,2,2-Trichloroethyl), Phenylphosphonate (LXXXV)

1 g of LXXXII (2.38 mmole) and 1.8 g of CsF (11.88 mmole) were dissolved in 17.4 ml of iso-propyl alcohol. As previously, the rate of the reaction was followed by g.l.c. (Table X). After 22.5 hr the reaction mixture was worked-up and purified as for the previous preparation. LXXXV was obtained as an oil in 59% yield (465 mg, 1.4 mmole).

PMR ($CDCl_3$, Fig. III): 7.2-8.0 ppm (5H, m, ϕ)

4.4-4.6 ppm (2H, d, $P-O-\underline{CH_2}-CCl_3$), $^2J_{P-O-CH_2} = 6$ Hz

4.6-5.0 ppm (1H, m, $-O-\underline{CH}(CH_3)_2$)

1.2-1.6 ppm (6H, t, $-O-CH(\underline{CH_3})_2$)

Ethyl, iso-Propyl, Phenylphosphonate (LXXXVII)

i) 465 mg of LXXXV (1.4 mmole) and 1.07 g of CsF (7 mmole) were dissolved in 10.4 ml of absolute ethanol. The solution was heated at 75°C and the speed of the reaction was checked by g.l.c. (Table X). After 10 hr, the solution was cooled down to room temperature and then left for 3 days. The reaction mixture was then worked-up as usual. The mixed dialkyl phenylphosphonate was kept under high vacuum for at

least 2 hr to remove last traces of 2,2,2-trichloroethanol. 287 mg of LXXXVII was obtained as an oil (1.26 mmole, 90%). The mass spectrum of the oily phosphonate showed a parent peak at m/e = 228.

PMR ($CDCl_3$, Fig. IV): 7.2-8.0 ppm (5H, m, ϕ)
4.4-5.0 ppm (1H, m, $-O-C\underline{H}(CH_3)_2$)
3.8-4.4 ppm (2H, q, $-O-C\underline{H_2}-CH_3$)
1.2-1.6 ppm (9H, t, $-O-CH(C\underline{H_3})_2$ and $-O-CH_2C\underline{H_3}$)

ii) 384 mg of LXXXIII (1.21 mmole) and 919 mg of CsF (6.05 mmole) were dissolved in 9 ml of iso-propanol. The solution was heated at 75°C. Table X indicates the kinetic course of the reaction (g.l.c.). After 10 hr the solution was cooled down to ambient temperature and then left for 3 days. After conventional work-up and high vacuum treatment, 253 mg of the oily phosphonate LXXXVII (1.11 mmole, 92%) was obtained. PMR spectrum was identical to the one described above.

5,5-Dimethyl-2-oxo-2-(2,2,2-trichloroethyl)-1,3,2-Dioxaphosphorinane (XC)

1 g of tris-(2,2,2-trichloroethyl) phosphate (LXXXVIII, 2.03 mmole), 4.23 g of 2,2-dimethyl-1,3-propanediol (LXXXIX, 40.7 mmole) and 3.08 g of CsF (20.3 mmole) were dissolved in

Table X. Fluoride Ion-Promoted Transesterification in Dialkyl Phenylphosphonates

ϕ-P(=O)(OR')(OR)	R"OH	T(°C)	Time(hr)	ϕ-P(=O)(OR')(OR) (%)	ϕ-P(=O)(OR")(OR') (%)	ϕ-P(=O)(OR")(OR") (%)
R=R'=TCE	R"=Et	25	12.5	16.0	79.7	4.3
"	"	"	15.5	7.5	85.8	6.7
"	"	"	19.5	4.8	87.7	7.4
"	"	"	22.5	3.2	86.7	10.1
"	"	"	24.0	100.0*	0.0	0.0
"	R"=i-Pr	"	12.5	10.3	88.1	1.6
"	"	"	15.5	6.0	92.0	2.0
"	"	"	19.5	2.8	95.0	2.2
"	"	"	22.5	1.9	95.8	2.3
"	"	"	24.0	100.0*	0.0	0.0
R=TCE						
R'=i-Pr	R"=Et	75	2.0	55.8	44.2	
"	"	"	5.0	17.1	82.9	
"	"	"	7.0	3.9	96.1	
"	"	"	10.0	2.6	97.4	
R=TCE						
R'=Et	R"=i-Pr	"	2.0	34.0	66.0	
"	"	"	5.0	13.5	86.5	
"	"	"	7.0	3.1	96.9	
"	"	"	10.0	2.3	97.7	

*Experiment carried out in the absence of cesium fluoride (CsF).

20 ml of dry tert-butanol. The solution was stirred at room temperature for 20 hr. G.l.c. indicated the presence of an intense new peak (> 95%) and the absence of starting material (LXXXVIII). The reaction mixture was concentrated under low pressure and the remaining material was subjected to standard extraction procedures. The cyclic phosphate triester was purified on silica gel plates which were developed three times with ether-hexanes (1:1). The band corresponding to XC was located upon treatment of the edge of a plate with granulated iodine. 361 mg of the triester (1.22 mmole, 60%) was isolated as a nice crystalline solid which was recrystallized from methanol, m.p. 127-128°C. The mass spectrum of XC exhibited a parent peak at m/e = 297.

PMR ($CDCl_3$, Fig. V): 4.5 ppm (2H, d, P-O-CH_2-CCl_3), $^2J_{P-O-CH_2}$ = 7 Hz
3.6-4.3 ppm (4H, (-O-CH_2-C)$_2$)
1.2 ppm (3H, S, CH_3)
0.8 ppm (3H, S, CH_3)

Alternate Preparation of XC

1 g of 5,5-dimethyl-2-oxo-2-phenoxy-1,3,2-dioxaphosphorinane[255] (XCI, 4.13 mmole) and 3.14 g of CsF (20.6 mmole) were dissolved in 20 ml of 2,2,2-trichloroethanol. The solution was heated at 80°C for 30 hr. G.l.c. showed two new peaks: The major (∿ 85%) corresponding to XC and the minor (∿ 10-15%), appearing at shorter retention time, probably

corresponding to the intermediate phosphorofluoridate XCII. Traces of starting material (< 5%) were still present. The reaction mixture was then worked-up and purified as for the previous preparation. XC was isolated in 55% yield (678 mg, 2.29 mmole). PMR's of XC from the two different preparations are perfectly superimposable.

5,5-Dimethyl-2-Fluoro-2-oxo-1,3,2-Dioxaphosphorinane (XCII) as a Possible Intermediate in the Fluoride Assisted Transesterification Reactions

Experiment #1: A test tube (10 ml) containing 100 mg of dry XCI (0.41 mmole) and 314 mg of CsF (2.07 mmole) was flushed with dry nitrogen through a serum cap. 2 ml of dry acetonitrile was added by syringe. The suspension was stirred at room temperature for 14 hr. Immediately after the stirring was stopped, an aliquot (3 µl) of the reaction mixture was taken through the septum for g.l.c. analysis. 91.2% of starting material (XCI) and 8.8% of the suspected phosphorofluoridate XCII were observed.

Experiment #2: Under identical conditions as for Experiment #1, 122 mg of XC (0.41 mmole) and 314 mg of CsF (2.07 mmole) were stirred at ambient temperature for 14 hr. As above, g.l.c. analysis showed 81.8% of starting material (XC) and 18.2% of phosphorofluoridate XCII having the same retention time as in Experiment #1 (Fig. VI).

bis-Methyl Ester of 5'-O-p-Monomethoxytrityl Thymidine-3'-Phosphate ($mmtTp^{Me}_{Me}$)

To 223 mg of anhydrous CsF (1.47 mmole) and 110 mg of $mmtTp^{\phi}_{\phi}$ (LXXIIa, 0.147 mmole) was added 5 ml of dry methanol. The solution was stirred at room temperature for 48 hr. The solvent was concentrated to a small volume and the material dissolved with $CHCl_3$ (10 ml). The solution was extracted with water (4 x 5 ml). Centrifugation was often necessary to break the emulsion formed during the extraction procedure. The organic layer was collected and evaporated under reduced pressure. The residue was then applied on preparative silica gel plates which were developed with Et_2O-EtOAc (1:1) and then with EtOAc. $mmtTp^{Me}_{Me}$ was isolated in 86% yield (79 mg, 0.127 mmole). Chromatographic and spectral properties of $mmtTp^{Me}_{Me}$ are given in Table XI.

bis-Ethyl Ester of 5'-O-p-Monomethoxytrityl Thymidine-3'-Phosphate ($mmtTp^{Et}_{Et}$)

To 223 mg of CsF (1.47 mmole) and 110 mg of $mmtTp^{\phi}_{\phi}$ (0.147 mmole) was added 5 ml of absolute ethanol. The solution was stirred at ambient temperature for 48 hr. The reaction mixture was then worked-up as above and purified on silica gel plates which were developed with Et_2O-EtOAc (1:1) and then with EtOAc. 84 mg of $mmtTp^{Et}_{Et}$ was obtained (0.129 mmole, 88% yield).

Table XI displayed the chromatographic and spectral properties of $mmtTp^{Et}_{Et}$.

Preparation of $mmtTp^{Et}_{Et}$ via an other route

514 mg of mmtT (1 mmole) was dried by coevaporation with dry pyridine (4 x 2 ml). 10 ml of dry pyridine was added followed by 0.24 ml of diethyl chlorophosphate (2 mmole). The solution was stirred at room temperature for 24 hr. 1 ml of water was added and the solution was evaporated under low pressure. The residue was dissolved in $CHCl_3$ (50 ml) and the solution was extracted once with a 10% aqueous solution of sodium bicaronate ($NaHCO_3$) (10 ml) and then, three times with water (10 ml). The chloroform layer was evaporated under reduced pressure. The residue was purified on silica gel plates as previously. The mononucleotide triester was isolated in 90% yield (585 mg, 0.9 mmole). Chromatographic and spectral properties are identical to those obtained from the previous preparation (Table XI).

bis-Butyl Ester of 5'-O-p-Monomethoxytrityl Thymidine-3'-Phosphate ($mmtTp^{Bu}_{Bu}$)

To 814 mg of CsF (5.36 mmole) and 400 mg of $mmtTp^{\phi}_{\phi}$ (0.534 mmole) was added 13 ml of dry n-butanol. The solution was stirred at ambient temperature for 48 hr. The reaction mixture

was then worked-up as usual and purified on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with ether. 332 mg of $mmtTp^{Bu}_{Bu}$ was obtained (0.470 mmole, 88% yield). After a standard detritylation and a further purification on silica gel plates, Tp^{Bu}_{Bu} was analyzed by mass spectrometry which gave a parent peak at m/e = 434. Usual chromatographic and spectral properties are listed in Table XI.

bis-(2-Chloroethyl) Ester of 5'-O-p-Monomethoxytrityl Thymidine-3'-Phosphate ($mmtTp^{CH_2CH_2Cl}_{CH_2CH_2Cl}$)

319 mg of CsF (2.1 mmole) and 157 mg of $mmtTp^{\phi}_{\phi}$ (0.21 mmole) were dissolved in 5 ml of dry 2-chloroethanol. The solution was stirred at ambient temperature for 48 hr. After conventional work-up and purification on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with ether, the transesterification product was isolated in 84% yield (127 mg, 0.177 mmole). Usual properties are given in Table XI.

Methyl, (2,2,2-Trichloroethyl) Ester of 5'-O-p-p'-Dimethoxytrityl Thymidine-3'-Phosphate ($dmtTp^{TCE}_{Me}$, CIIa)

304 mg of CsF (2 mmole) and 177 mg of $dmtTp^{TCE}_{TCE}$ (0.20 mmole) were dissolved in 5 ml of dry methanol. The solution was stirred at 50-55°C for 17.5 hr. The solution was then

concentrated to a small volume (1-2 ml) under low pressure. The concentrate was poured onto ice water (25 ml). The reaction flask was rinsed with the minimum amount of methanol (1 ml) which was then added to the cold aqueous suspension. The latter was filtered through a glass sintered funnel. The white precipitate was dissolved in chloroform and then applied on silica gel plates which were developed with Et_2O-hexanes (3:1) and then twice with Et_2O. 109 mg of $dmtTp^{TCE}_{Me}$ was isolated (0.14 mmole, 71% yield) as a white solid m.p. 83-88 °C from precipitation with hexanes. Properties from further characterization are listed in Table XI.

Ethyl (2,2,2-Trichloroethyl) Ester of 5'-O-p-p'-Dimethoxytrityl Thymidine-3'-Phosphate ($dmtTp^{TCE}_{Et}$, CIIb)

304 mg of CsF (2 mmole) and 177 mg $dmtTp^{TCE}_{TCE}$ (0.2 mmole) were dissolved in absolute ethanol. The solution was stirred at 50-55°C for 8.5 hr. The reaction mixture was then worked-up as for the previous preparation. The purification of the reaction products was achieved on silica gel plates which were developed with Et_2O-hexanes (3:1) and twice with ether. CIIb was obtained in 75% yield (119 mg, 0.15 mmole) as a nice solid m.p. 78-83 °C from precipitation with hexanes. Table XI gave information concerning the chromatographic and spectral properties of this compound.

Butyl (2,2,2-Trichloroethyl) Ester of 5'-O-p-p'-Dimethoxytrityl Thymidine-3'-Phosphate ($dmtTp^{TCE}_{Bu}$, CIIc)

To 304 mg of CsF (2 mmole) and 177 mg of $dmtTp^{TCE}_{TCE}$ (0.2 mmole) was added 5 ml of n-butanol. The solution was stirred at room temperature for 48 hr. The reaction mixture was evaporated under low pressure and then coevaporated twice with ethanol. The nucleotidic material was dissolved in the minimum amount of methanol (1-2 ml) and was then poured into ice cold water (25 ml) as for the previous preparations. The nucleotidic material was applied on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. CIIc was obtained from the usual precipitation from hexanes as a solid in 75% yield (120 mg, 0.15 mmole) m.p. 76-81 °C. The triester was further characterized and its properties are given in Table XI.

Methyl Phosphate Ester of 5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine (mmtTp(Me)TAc)

191 mg of CsF (1.26 mmole) and 118 mg of mmtTp(ϕ)TAc (0.126 mmole) were dissolved in 3 ml of dry methanol. The solution was stirred at ambient temperature for 43 hr. The reaction mixture was then worked-up as for the preparation of $mmtTp^{Me}_{Me}$. The purification of the dinucleotide triester was accomplished on silica gel plates which were developed with EtOAc and then with EtOAc-THF (1:1)). 93 mg of mmtTp(Me)TAc

was obtained after precipitation with hexanes (0.106 mmole, 84% yield) as a white powder m.p. 123-128°C. Chromatographic and spectral properties of the dinucleotide triester are given in Table XI.

Ethyl Phosphate Ester of 5'-O-Trityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine (trTp(Et)TAc)

574 mg of CsF (3.78 mmole) and 342 mg of trTp(ϕ)TAc (0.378 mmole) were dissolved in 9 ml of absolute ethanol. The solution was stirred at ambient temperature for 29 hr. After usual work-up and purification on silica gel plates which were developed with Et_2O and twice with EtOAc, 256 mg of trTp(Et)TAc (0.298 mmole, 79% yield) was obtained as a nice solid m.p. 116-121°C. Plates were further developed with EtOAc-THF (1:1) and 23 mg of trTp(Et)T was isolated (0.029 mmole, 7%). The total yield of the transesterification reaction was therefore 86%. Properties of these phosphotriesters are listed in Table XI.

Butyl Phosphate Ester of 5'-O-Trityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine (trTp(Bu)TAc)

191 mg of CsF (1.26 mmole) and 114 mg of trTp(ϕ)TAc (0.126 mmole) were dissolved in 3 ml of n-butanol. The solution was stirred at room temperature for 48 hr. The reaction mixture

was then worked-up and purified on silica gel plates which were developed with Et_2O and then with EtOAc. 78 mg of trTp(Bu)TAc was obtained (0.09 mmole, 70% yield) as a solid, m.p. 112-117°C. Plates were further developed with EtOAc-THF (1:1) and trTp(Bu)T was isolated in 8% yield (9 mg, 0.01 mmole) raising to 78% the overall transesterification yield. Table XI displayed the chromatographic and spectral properties of trTp(Bu)TAc.

General Procedure for the Deprotection of Nucleotide Triesters by Fluoride Ion in tert-Butanol, 2-Cyanoethanol and 2-Trimethylsilylethanol

0.02 mmole of the nucleotidic material ($dmtTp^{\phi}_{\phi}$, $dmtTp^{TCE}_{TCE}$ and mmtTp(ϕ)TSi and 0.2 mmole of CsF were dissolved in the appropriate alcohol. The reaction mixtures were stirred at room temperature for 30 hr for those using $dmtTp^{\phi}_{\phi}$ as starting material, and 50 hr for those using $dmtTp^{TCE}_{TCE}$ or mmtTp(ϕ)TSi as starting material. For a reaction using tert-butanol or 2-trimethylsilylethanol as solvent, the solution was evaporated under low pressure, the residue was dissolved with 80% aqueous acetic acid (10-15 ml) and the resulting solution was stirred at 85°C for 15-30 min. For a reaction using 2-cyanoethanol as solvent, 10 ml of $CHCl_3$ was added and the solution was extracted once with 5 ml of water. The organic layer was evaporated under reduced pressure. The residue was then treated with acetic acid as above. In all cases, excess acetic

Table XI. Thin-Layer Chromatographic and Spectral Data of Transesterification Products

Compound	Rf Values			$\lambda^{EtOH\ 95\%}_{max}$ (nm)	$\lambda^{EtOH\ 95\%}_{min}$ (nm)
*mmtTp$^{\phi}_{\phi}$	0.14[a]	0.46[b]	0.77[c]	266,263	248
mmtTp$^{Me}_{Me}$	0.08[c]	0.23[d]	0.68[e]	265	254
mmtTp$^{Et}_{Et}$	0.09[b]	0.22[c]	0.30[d]	265	245
mmtTp$^{Bu}_{Bu}$	0.14[a]	0.42[b]	0.61[c]	266	249
mmtTp$^{CH_2CH_2Cl}_{CH_2CH_2Cl}$	0.20[c]	0.45[d]	0.79[e]	266	247
*Tp$^{\phi}_{\phi}$	0.08[b]	0.33[c]	0.44[d]	266,263	234
Tp$^{Me}_{Me}$	0.03[d]	0.19[e]	0.38[f]	265	233
Tp$^{Et}_{Et}$	0.05[d]	0.33[e]	0.55[f]	265	234
Tp$^{Bu}_{Bu}$	0.03[a]	0.11[b]	0.20[c]	265	233
Tp$^{CH_2CH_2Cl}_{CH_2CH_2Cl}$	0.03[c]	0.10[d]	0.55[e]	266	234
*dmtTp$^{TCE}_{TCE}$	0.14[a]	0.48[b]	0.78[c]	266	254
dmtTp$^{TCE}_{Me}$	0.02[a]	0.11[b]	0.58[c],0.49[c] (Stereoisomers)	267	254
dmtTp$^{TCE}_{Et}$	0.05[a]	0.20[b]	0.60[c]	266	254
dmtTp$^{TCE}_{Bu}$	0.11[a]	0.38[b]	0.72[c]	267	254
*Tp$^{TCE}_{TCE}$	0.13[b]	0.42[c]	0.55[d]	264	234
Tp$^{TCE}_{Me}$	0.03[b]	0.13[c]	0.20[d]	265	234
Tp$^{TCE}_{Et}$	0.04[b]	0.15[c]	0.23[d]	264	234
Tp$^{TCE}_{Bu}$	0.10[b]	0.29[c]	0.39[d]	265	234
*trTp(ϕ)TAc	0.02[b]	0.18[c]	0.42[d]	267	246
mmtTp(Me)TAc	0.02[d]	0.46[e]	0.79[f]	266	246

Table XI continued

trTp(Et)TAc	0.13[d]	0.71[e]	0.86[f]	264	240
trTp(Bu)TAc	0.08[c]	0.20[d]	0.71[e]	265	246
trTp(Et)T	0.03[d]	0.50[e]	0.84[f]	265	240
trTp(Bu)T	0.07[d]	0.53[e]	0.71[f]	265	246
Tp(Me)TAc	0.12[e]	0.43[f]	0.35[g]	265	234
Tp(Bu)TAc	0.54[e]	0.80[f]	0.60[g]	265	234
Tp(Et)T	0.14[e]	0.64[f]	0.46[g]	265	234
Tp(Bu)T	0.22[e]	0.65[f]	0.47[g]	265	234
trTp($(CH_2)_4$OH)TAc	0.02[c]	0.05[d]	0.57[e]	264	240
trTp($(CH_2)_4$OSi)TAc	0.03[b]	0.26[c]	0.52[d]	265	240
Tp($(CH_2)_4$OH)T (CVIIa)	0.08[e]	0.54[f]	0.34[g]	265	234
SiTp($(CH_2)_4$OSi)TSi (CVIIb)	0.16[b]	0.74[d]	0.62[c]	265	234
CVIIIa	0.00[c]	0.02[d]	0.71[e]	264	240
CVIIIb	0.03[e]	0.57[f]	0.21[g]	265	234
CVIIIc	0.13[d]	0.61[h]	0.92[e]	265	234

*Added for comparison.

Solvent system: a = Et_2O-hexanes (3:1); b = Et_2O; c = Et_2O-EtOAc (1:1); d = EtOAc; e = EtOAc-THF (1:1); f = THF; g = $CHCl_3$:MeOH (8:2); h = EtOAc-THF (4:1).

acid was removed under low pressure and the nucleotidic material was applied on preparative papers which were developed in solvent B' (mononucleotide) or solvent A (dinucleotide). Bands were eluted from paper with water and their yields determined spectrophotometrically. Results are given in Table II.

Phenyl Phosphate Ester of 5'-O-Trityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine (trTp(ϕ)TAc, CIII)

In a 25 ml round bottom flask which was flushed with dry nitrogen through a serum cap, were syringed 1.18 ml of collidine (8.92 mmole) and 6 ml of dry THF. The solution was cooled to -78° with a dry ice iso-propanol bath. 320 µl of phenyldichlorophosphite[244] ($\phi OPCl_2$, 2.23 mmole) was added. A solution of 1.25 g of trT (2.23 mmole) in 3 ml of dry THF was immediately added dropwise over a period of 12 min. 15 min after completion of addition, a solution of 507 mg of 3'-O-acetyl thymidine (1.78 mmole) in 2 ml of dry THF and 2 ml of dry DMF was also added dropwise, over a period of 5 min. It is important to note that the tubes containing the dry nucleosides were stoppered with serum caps and were flushed with dry nitrogen before addition of the solvent. The reaction mixture was stirred at -78°C for a further 45 min and then allowed to warm up to room temperature for 1 hr. 930 mg of iodine (3.66 mmole) in a solution composed of 4 ml of THF,

2 ml of water and 1 ml of pyridine, was added to the reaction mixture. The red-brown solution was stirred at room temperature for 15 min. The solvent was then removed under reduced pressure. The material was dissolved in $CHCl_3$ (40 ml) and shaken with 20 ml of water and 10 ml of a freshly prepared 10% aqueous sodium bisulfite solution. The slightly yellow organic layer was collected and the aqueous phase was extracted twice with $CHCl_3$ (20 ml). The combined chloroform fractions were concentrated under vacuo and the nucleotidic material was applied on silica gel plates which were developed with Et_2O and then twice with EtOAc. 906 mg of trTp(ϕ)TAc was isolated (1.00 mmole, 56% yield) as a solid after precipitation with hexanes m.p. 112-117°C. Properties from further characterization of this triester are given in Table XI.

Attempted Synthesis of 2-Hydroxyethyl Phosphate Ester of 5'-O-Trityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine (CVa)

566 mg of trTp(ϕ)TAc (0.625 mmole) and 950 mg of CsF (6.25 mmole) were suspended in 15 ml of dry ethylene glycol. DMF was added dropwise until a clear solution was formed. The reaction mixture was stirred at ambient temperature for 49 hr. The solution was then poured into ice-water (150 ml). The precipitate was collected by filtration and centrifugation, dissolved in $CHCl_3$ and then purified on silica gel plates which were developed with Et_2O and then with EtOAc. 233 mg of pure

trT (0.482 mmole, 77% yield) was isolated. Its chromatographic properties are identical to those of an authentic sample in two different solvents. Furthermore, the reaction product was detritylated by treatment with 80% aqueous acetic acid (85°C, 30-45 min) and was then spotted on paper chromatography beside an authentic thymidine sample. In three different solvents (A, B' and F) the reaction product moved identically as thymidine.

Plates were developed further with EtOAc-THF (1:1) affording trTp(ϕ)T in 17% yield (92 mg, 0.106 mmole). Its chromatographic properties on TLC are identical to those of an authentic sample.

4-Hydroxybutyl Phosphate Ester of 5'-O-Trityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine (CVb)

320 mg of trTp(ϕ)TAc (0.353 mmole) and 536 mg of CsF (3.53 mmole) were suspended in 6 ml of dry 1,4-butanediol. Dry THF was added until a clear solution was formed (1-2 ml). The solution was stirred at room temperature for 29 hr. The reaction mixture was then poured into ice water (100 ml). Most of the precipitate was isolated by filtration through a glass sintered funnel (coarse porosity). The filtrate, appearing as a fine suspension, was then frozen and allowed to melt slowly. After filtration, the aqueous solution was almost perfectly clear. The combined precipitates were

dissolved in $CHCl_3$ (60 ml) and the solution was extracted once with water (20 ml). The organic layer was collected and evaporated under low pressure. The material was applied on silica gel plates which were developed with EtOAc and then with EtOAc-THF (1:1). CVb was obtained in 78% yield (249 mg, 0.276 mmole) as a solid from precipitation with hexanes, m.p. 122-127°C. The deacetylated derivative CVIb was isolated in 8% yield (24 mg, 0.028 mmole). CVb was fully deprotected by successive treatment with concentrated ammonium hydroxide in pyridine (1:1) for 15 hr at room temperature and with 80% aqueous acetic acid at 85°C for 45 min. After purification on TLC plates, the fully deprotected dinucleotide triester (CVIIa) was characterized by CMR. CVIIa was fully silylated according to Ogilvie's method[173] and this derivative was also characterized by CMR. Chemical shifts are reported in Table III. Table XI showed the chromatographic and spectral properties of these phosphotriesters.

Preparation of the Tetranucleotide Triester Bridged Through Phosphate Moieties (CVIIIa)

958 mg of CVb (1.06 mmole), 4.81 g of trTp(ϕ)TAc (CIII, 5.31 mmole) and 1.61 g of CsF (10.6 mmole) were dissolved in a solution of 29 ml of dry tert-butanol and 29 ml of dry DMF. The solution was stirred at room temperature for 61.5 hr. The solvent was removed under low pressure (T < 45°C)

and the material was dissolved in $CHCl_3$ (100 ml). The solution was extracted with water (4 x 25 ml). Centrifugation was often required for breaking emulsions. The organic layer was concentrated under vacuum and the concentrate was applied to a dry column of silica gel (7 cm x 9 cm) which was washed successively with hexanes, Et_2O and EtOAc. The reaction products were eluted from the column with THF. Excess dinucleotide diester remained at the top of the column. Attempts to separate on silica gel plates, the reaction product (Rf(EtOAc-THF (1:1)) = 0.71) from the starting material (Rf(EtOAc-THF (1:1)) = 0.57) failed. Therefore, the mixture was reacted with 308 mg of TBDMSCl (2.04 mmole) and 330 mg of imidazole (4.85 mmole) in 2 ml of dry DMF. After 15 hr at room temperature, the solution was poured into 150 ml of ice cold-water. After filtration, the silylated compounds were separated on silica gel plates which were developed once with Et_2O and twice with EtOAc. 223 mg of the silylated starting material CVIIb (0.219 mmole, 21%) was isolated. Plates were developed further with EtOAc-THF (1:1) affording 538 mg of the reaction product CVIIIa (0.314 mmole, 30% yield) untouched by the silylation reaction. CVIIIa was obtained as a nice powder m.p. 153-158°C from precipitation with hexanes.

Mol. wt. (Galbraith Laboratories) calcd. for $C_{86}H_{92}O_{26}N_8P_2$: 1714

Found: 1728

A test reaction involving CVb (25 mg, 0.028 mmole) and CsF (42 mg, 0.278 mmole) in the absence of CIII was carried out in 0.75 ml of dry tert-butanol and 0.75 ml of dry DMF. As above, the reaction mixture was stirred at room temperature for 67 hr and was similarly worked-up thereafter. TLC failed to indicate a reaction product moving slightly faster than starting material in EtOAc-THF (1:1). However, loss of 3'-acetyl group was observed (< 10%) as well as some starting material degradation product at the base line (< 10%).

CVIIIa was fully deprotected under similar conditions as for CVb. The fully deprotected CVIIIb was fully silylated to CVIIIc according to Ogilvie's procedure[173]. Both CVIIIb and CVIIIc were characterized by CMR and their chemical shifts are given in Table III.

The chromatographic and spectral properties of the "bridged" tetranucleotide triesters are given in Table XI.

Acylation and alkylation of nucleosides and nucleotides using tetrabutyl ammonium fluoride.

5'-O-p-Monomethoxytrityl-3'-O-Pivaloyl Thymidine (CXb)

To 978 mg of pivalic anhydride (CIXb, 5.25 mmole) in 5 ml of dry THF, was added 108 mg of mmtT (LXVb, 0.21 mmole) or 132 mg of its 3'-O-TBDMS derivative (LXVc, 0.21 mmole) followed by 3.1 ml of a solution of 0.68 M TBAF (2.1 mmole).

The solution was stirred at room temperature for 24 hr. Excess THF was removed under reduced pressure (T < 50°C) and the residue was dissolved in $CHCl_3$ (10 ml). The solution was then extracted with a 10% aqueous sodium bicarbonate ($NaHCO_3$) solution (4 x 5 ml) and then with water (4 x 5 ml). The organic layer was evaporated to dryness under low pressure. The material was dissolved with the minimum of $CHCl_3$ and then applied on two silica gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O-hexanes (3:1). Bands were eluted from silica gel with ethyl acetate. When mmtT was used as starting material, the minimum time for a complete reaction was 5 hr and 128 mg of CXb (0.21 mmole) was isolated (100% yield). When LXVc was used as starting material, the minimum time for a complete reaction was 12 hr and CXb was obtained in 95% yield (119 mg, 0.20 mmole); m.p. 102-104°C. Chromatographic and ultraviolet properties of CXb are given in Table XII. An identical scale reaction was carried out using mmtT as starting material and in which TBAF and THF were replaced by 5 ml of dry pyridine. After 24 hr at room temperature, the reaction mixture was worked-up as above and then applied on silica gel plates which were developed with Et_2O-hexanes (3:1) and Et_2O. LXVb was recovered in 91% yield (98 mg, 0.19 mmole). CXb accounted for 9% of all the nucleosidic material present.

Similarly, a solution of mmtT (0.21 mmole) and 0.65 ml

of pivaloyl chloride (5.25 mmole) in 5 ml of dry pyridine was stirred at room temperature for 24 hr.

After usual work-up and purification on silica gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O-hexanes (3:1), two bands were observed and isolated. The minor band contained 28 mg of CXb (0.047 mmole, 22%; Rf: 0.40 (Et_2O-hexanes (3:1)); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 249 nm. The major band accounted for 78% of the nucleosidic material present and proved to have an extra pivaloyl group at the N^3-position[196] as evidenced by mass spectral analysis performed on N^3-pivaloyl-3',5'-O-pivaloyl thymidine which was similarly prepared. N^3-pivaloyl-5'-O-p-monomethoxytrityl-3'-O-pivaloyl thymidine has the following properties: Rf: 0.65 (Et_2O-hexanes (3:1)); u.v. (95% EtOH) λ_{max} = 269 nm, λ_{min} = 250 nm; m.p. 63-65 °C.

5'-O-p-Monomethoxytrityl-3'-O-Acetyl Thymidine (CXa)

To 0.5 ml of distilled acetic anhydride (CIXa, 5.25 mmole) in 5 ml of dry THF was added 108 mg of mmtT (LXVb, 0.21 mmole) or 132 mg of mmtTSi (LXVc, 0.21 mmole) followed by 3.1 ml of 0.68 M TBAF (2.1 mmole). The solution was stirred at room temperature for 24 hr. The reaction mixture was then worked-up as for the previous preparations. Purification of the reaction mixture was done on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. With

LXVb as starting material, 116 mg of mmtTAc (CXa) was isolated (0.208 mmole, 99%); m.p. 80-84°C. The minimum time required for a complete reaction was 30 min. With LXVc as starting material, 110 mg of CXa was obtained (0.20 mmole, 94%) and the minimum time required for a complete reaction was 12 hr. Table XII displayed chromatographic and spectral data of mmtTAc.

Using LXVb (0.21 mmole) in the absence of TBAF under the above conditions produced 9 mg of mmtTAc (0.017 mmole, 8%) after 24 hr along with 99 mg of starting material LXVb (0.193 mmole, 92%). Similarly, the reaction of mmtT (0.21 mmole) with acetic anhydride (5.25 mmole) in 5 ml of dry pyridine afforded after 24 hr at room temperature 94 mg of CXa (0.169 mmole, 80%); Rf: 0.49 (Et_2O); u.v. (95% EtOH) λ_{max} = 266 nm, λ_{min} = 251 nm. N^3-acetyl-5'-O-p-monomethoxytrityl-3'-O-acetyl thymidine accounted for 20% of all the nucleosidic material present in the reaction mixture; Rf: 0.72 (Et_2O); u.v. (95% EtOH) λ_{max} = 269 nm, λ_{min} = 249 nm.

5'-O-p-Monomethoxytrityl-3'-O-Benzoyl Thymidine (CXc)

To 1.19 g of benzoic anhydride (CIXc, 5.25 mmole) in 5 ml of dry THF was added 108 mg of mmtT (LXVb, 0.21 mmole) or 132 mg of mmtTSi (LXVc, 0.21 mmole). 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at room temperature for 24 hr. 2-3 ml of water was then added to the solution which was stirred at ambient temperature for 15 hr.

After conventional work-up, the reaction mixture was purified on silica gel plates which were developed twice with Et_2O-hexanes (3:1). 127 mg of mmtTBz (CXc) was obtained (0.205 mmole, 98%); m.p. 104-107°C. The minimum time required for a complete reaction was 2 hr. When the starting material was LXVc, 126 mg of mmtTBz was isolated (0.204 mmole, 97%). 15 hr is the minimum time required for a complete reaction. Chromatographic and spectral properties of CXc are given in Table XII.

An identical scale reaction using mmtT and benzoic anhydride in dry THF was carried out at ambient temperature for 24 hr. After usual work-up and purification on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. 47 mg of mmtTBz was obtained (0.076 mmole, 36%) along with 69 mg of mmtT (0.134 mmole, 64%).

LXVb (0.21 mmole) and CIXc (5.25 mmole) were dissolved in 5 ml of dry pyridine. The solution was stirred at ambient temperature for 24 hr. After usual work-up and purification on silica gel plates, 85 mg of mmtTBz was isolated (0.137 mmole, 65%). Starting material (LXVb) accounted for all the remaining material (35%). No acylation at the nucleoside base was observed in this case.

5'-O-p-Monomethoxytrityl-3'-O-Myristoyl Thymidine (CXd)

108 mg of mmtT (LXVb, 0.21 mmole) or 132 mg of mmtTSi

(LXVc, 0.21 mmole) and 2.3 g of myristic anhdride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the reaction mixture was stirred at ambient temperature for 24 hr. After a difficult work-up requiring centrifugation for breaking the emulsions, purification of the reaction products was achieved using silica gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O-hexanes (3:1). 84 mg of mmtTMyr was isolated as a gum (0.116 mmole, 55%). Starting material (LXVb) accounted for all the remaining material (45%). The minimum time required for a complete reaction was about 8 hr. 97 mg of a gummy CXd was obtained (0.134 mmole, 64%) when the original reaction mixture was heated at reflux for 4 hr. When LXVc was used as starting material, 60 mg of mmtTMyr was obtained (0.083 mmole, 40%) leaving 60% of unreacted mmtT. The minimum time required for a complete reaction was about 15 hr. However, upon reluxing for 4 hr, 73 mg of CXd was obtained (0.101 mmole, 48%) leaving 52% of untouched mmtT. Table XII showed the chromatographic and ultraviolet properties of CXd.

The reaction of mmtT (0.21 mmole) and myristic anhydride (5.25 mmole) in 5 ml of dry pyridine for 24 hr afforded after a difficult work-up and purification on silica gel plates, 64 mg of mmtTMyr (0.088 mmole, 42%). No acylation at the nucleoside base was observed on TLC.

2',5'-di-O-Trityl-3'-O-Pivaloyl Uridine (CXII)

309 mg of 2,5-di-O-trityl uridine (CXI, 0.42 mmole) was dissolved in 6.2 ml of 0.68 M TBAF. 1.96 g of pivalic anhydride (CIXb, 10.5 mmole) was added and the solution was heated at 85°C for 13 hr. Excess THF was removed under low pressure and the residue was dissolved in $CHCl_3$ (30 ml). The solution was extracted with water (4 x 15 ml) and then with a 10% aqueous solution of ammonium hydroxide (2 x 15 ml). Subsequently, the organic layer was washed with water (2 x 15 ml). The organic phase was collected and then evaporated under reduced pressure. The mixture was applied to four silica gel plates which were developed with Et_2O-hexanes (1:1) and Et_2O-hexanes (3:1). A moving band and a base line band were eluted from silica gel with methanol. After evaporation of the solvent, TLC showed that the compounds corresponding to both bands were identical. 327 mg of CXII was isolated (0.40 mmole, 96%). CXII was recrystallized from benzene-pentane affording white crystalline material melting at 254-256°C; Rf: 0.56 (Et_2O-hexanes (3:1)); u.v. (95% EtOH) λ_{max} = 260 nm, λ_{min} = 244 nm.

PMR ($CDCl_3$, Fig. VII): 7.2 ppm (30H, S, $-C(\phi)_3$)

1.2 ppm (9H, S, $-C(CH_3)_3$)

A test reaction indicated that the minimum time required for a complete reaction was about 3 hr.

A small scale reaction using 31 mg of CXI (0.042 mmole) and 196 mg of pivalic anhydride (1.05 mmole) was carried out in dry pyridine (1 ml) as solvent in the absence of TBAF. The solution was heated at 85°C for 24 hr. After identical work-up as above, TLC showed the presence of starting material Rf: 0.13 (Et_2O-hexanes (3:1) as the major compound (> 90%). Traces of the desired product (CXII, < 5%) along with a material (< 5%) moving faster than CXII were also observed.

N-Benzoyl-3',5'-di-O-Benzoyl Deoxycytidine ($BzdC^{Bz}Bz$)

48 mg of deoxycytidine (0.21 mmole) and 1.19 g of benzoic anhydride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at ambient temperature for 24 hr. 2-3 ml of water was then added and the reaction mixture was stirred at room temperature for a further 15 hr. After usual extraction procedure, the reaction products were purified on two silica gel plates which were developed with Et_2O and then with EtOAc. $BzdC^{Bz}Bz$ was obtained as a white powder m.p. 177-180°C. Yield: 102 mg (0.189 mmole, 90%). The chromatographic properties of the reaction product were identical to those of an authentic sample synthesized by Khorana's procedure[172] (Table XII).

3',5'-di-O-Benzoyl Deoxyadenosine (BzdABz)

53 mg of deoxyadenosine (0.21 mmole) and 1.19 g of benzoic anhydride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at room temperature for 24 hr. The reaction mixture was then worked up as for the previous preparation. Purification of the reaction product was done on silica gel plates which were developed with Et_2O, EtOAc and finally EtOAc-THF (1:1). 20 mg of a minor compound which appeared to be N^6-benzoyl-3',5'-di-O-benzoyl deoxyadenosine was isolated (0.035 mmole, 17%). Its ultraviolet properties (Table XII) are in good agreement with those reported by Smrt and coworkers[46] for N^6-benzoyl-2',3'-isopropylidene adenosine suggesting that the reaction product has a N^6-benzoyl group. 75 mg of the major reaction product (BzdABz) was isolated (0.163 mmole, 78%) as a white powder; m.p. 113-114°C; m/e = 459. Ultraviolet and chromatographic properties of BzdABz are reported in Table XII.

3',5'-di-O-Benzoyl Deoxyguanosine (BzdGBz)

56 mg of deoxyguanosine (0.21 mmole) and 1.19 g of benzoic anhydride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at ambient temperature for 24 hr. After

usual aqueous treatment and work-up, the reaction mixture was purified on silica gel plates which were developed with THF and then with $CHCl_3$-MeOH (8:2). 81 mg of BzdGBz was obtained (0.17 mmole, 81%); m.p. 175-176°C. N^2-benzoyl-3,5'-di-O-benzoyl deoxyguanosine ($BzdG^{Bz}Bz$) was prepared according to Khorana's procedure[172]. $BzdG^{Bz}Bz$ moved faster than BzdGBz on TLC in three different solvents. N^2-benzoyl deoxyguanosine (dG^{Bz}) was prepared from $BzdG^{Bz}Bz$ by treatment with an aqueous solution of 2N sodium hydroxide for 5 min at room temperature[172]. BzdGBz moved faster than dG^{Bz} on TLC in three different solvents. Finally, BzdGBz was hydrolyzed under the alkaline conditions used for $BzdG^{Bz}Bz$ and as expected deoxyguanosine (dG) was produced suggesting that the proposed structure is correct. Chromatographic and spectral properties of these guanosine derivatives are reported in Table XII.

3',5'-di-O-Acetyl Thymidine (CXIVa)

100 mg of CXIII (SiTSi, 0.21 mmole) and 0.5 ml of distilled acetic anhydride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF was added and the solution was stirred at room temperature for 24 hr. After a conventional work-up, the reaction mixture was purified on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. 66 mg of CXIVa was obtained (0.202 mmole, 97%); m.p. 124-125°C. The minimum time required for a

complete reaction was 9 hr. Table XII showed the chromatographic and ultraviolet properties of CXIVa.

A test reaction was carried out under the above conditions in the absence of TBAF. No reaction occurred at all even after 24 hr.

Attempted Preparation of 3',5'-di-O-Acetyl Thymidine Via the Ferric Chloride/Acetic Anhydride Reaction[198]

100 mg of CXIII (0.21 mmole) was dissolved in 1 ml of EtOAc and 2 ml of distilled acetic anhydride. 10 mg of anhydrous ferric chloride (0.06 mmole) was added and the brown solution was stirred at room temperature for 40 hr. The solvent was evaporated under low pressure and the residue was dissolved in chloroform (10 ml). The solution was then extracted with water (4 x 3 ml). The organic layer was collected and evaporated under low pressure. The residue was purified on silica gel plates which were developed with Et_2O-hexanes (3:1) and then 3 times with Et_2O. The major compound was isolated and exhibited the following properties with respect to AcTAc on silica gel and paper chromatography:

Compound	Rf^{Et_2O}	$Rf^{B'}$	Rf^{A}	$\lambda_{max}^{EtOH\ 95\%}$ (nm)	$\lambda_{min}^{EtOH\ 95\%}$ (nm)
AcTAc	0.22	0.73	0.80	265	234
Reaction product	0.23	0.82	0.68	262	233

PMR ($CDCl_3$) of the reaction product showed two broad singlets at 2.1-2.2 ppm integrating for four acetyl groups compared with one singlet at 2.2 ppm corresponding to the two acetyl groups in the standard diacetate CXIVa. Mass spectrometry revealed the parent peak of the reaction product at m/e = 428 suggesting that the tetraacetate structure, CXV is correct. 68 mg of CXV was isolated (0.159 mmole, 76%); m.p. 109-111°C.

The tetraacetate CXV was deprotected with a solution of concentrated ammonium hydroxide in pyridine (1:1) for 24 hr. The resulting tetra-ol was spotted beside thymidine on papers which were developed with solvent A, B' and F. Results are given below.

Compound	$Rf^{B'}$	Rf^{A}	Rf^{F}	$\lambda_{max}^{EtOH\ 95\%}$ (nm)	$\lambda_{min}^{EtOH\ 95\%}$ (nm)
tetra-ol derivative of CXV	0.55	0.63	0.79	263	233
T	0.56	0.69	0.80	265	234

3',5'-di-O-Pivaloyl Thymidine (CXIVb)

To 978 mg of pivalic anhydride (5.25 mmole) in 5 ml of dry THF, was added 100 mg of SiTSi (0.210 mmole) followed by 3.1 ml of 0.68 M TBAF. The solution was stirred at ambient temperature for 24 hr. After a typical work-up, the reaction mixture was purified on silica gel plates which were developed 3 times with Et_2O-hexanes (3:1). 83 mg of PvTPv was obtained

(0.202 mmole, 96%) and was recrystallized from Et_2O-hexanes. The white needles melted at 120-121°C. The mass spectrum displayed a parent peak at m/e = 410. The minimum time required for a complete reaction was about 9 hr. Chromatographic and spectral properties of CXIVb are given in Table XII.

N^3-Pivaloyl-3',5'-di-O-Pivaloyl Thymidine

51 mg of thymidine was dissolved in 5 ml of dry pyridine. 0.65 ml of pivaloyl chloride (1.05 mmole) was added and the solution was stirred at room temperature for 24 hr. After usual work-up the reaction mixture was purified on silica gel plates which were developed twice with Et_2O-hexanes (1:1). 81 mg of the titled compound was isolated (0.16 mmole, 76%) m.p. 104-106°C; Rf: 0.46 (Et_2O-hexanes (1:1)); u.v. (95% EtOH) λ_{max} = 267 nm, λ_{min} = 236 nm. The mass spectrum displayed a parent peak at m/e = 494. 8 mg of CXIVb was also obtained (0.019 mmole, 9%); Rf: 0.15 (Et_2O-hexanes (1:1)); u.v. (95% EtOH) λ_{max} = 265 nm, λ_{min} = 233 nm. 6 mg of an unidentified material; Rf: 0.30 (Et_2O-hexanes (1:1)); u.v. (95% EtOH) λ_{max} = 267 nm, λ_{min} = 236 nm was isolated as well.

3',5'-di-O-Pivaloyl Thymidine (PvTPv) Via the Ferric Chloride/ Pivalic Anhydride Reaction

100 mg of SiTSi (0.21 mmole) was dissolved in 1 ml of

of EtOAc and 2 ml of pivalic anhydride. 15 mg of anhydrous ferric chloride was added (0.09 mmole) and the brown solution was stirred at room temperature for 40 hr. After a work-up identical as for the tetraacetate preparation, the reaction products were purified on silica gel plates which were developed three times with Et_2O-hexanes (3:1). 53 mg of PvTPv was obtained as a white foam (0.13 mmole, 62%). Mass spectrometry showed the parent peak at m/e = 410. The acylated product was treated with a solution of 0.2 N sodium hydroxide for 7 hr at room temperature. The solution was then neutralized with DOWEX 50W-X8 pyridinium form ion exchange resin. After evaporation of water under reduced pressure, the residue was spotted on papers which were developed with solvents A and B'. In both solvents, the reaction product moved the same as thymidine.

3',5'-di-O-Benzoyl Thymidine (CXIVc)

100 mg of SiTSi (0.21 mmole) and 1.19 g of benzoic anhydride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at room temperature for 24 hr. 2-3 ml of water was added and the solution was further stirred at ambient temperature for 15 hr. After conventional work-up the reaction mixture was applied on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. 93 mg

of BzTBz was isolated (0.206 mmole, 98%) as a white solid m.p. 195-196°C. A test reaction indicated that 9 hr is the minimum time required for a complete reaction. Table XII displayed the ultraviolet and chromatographic properties of CXIVc.

3',5'-di-O-Myristoyl Thymidine (CXIVd)

100 mg of SiTSi (0.21 mmole) and 2.3 g of myristic anhydride (5.25 mmole) were dissolved in 5 ml of dry THF. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at room temperature for 24 hr. 3 ml of water and 3 ml of pyridine were then added and the solution was stirred at ambient temperature for 15 hr. After usual work-up the reaction products were purified on silica gel plates which were developed with Et_2O-hexanes (1:1) and then with Et_2O-hexanes (3:1). 68 mg of MyrTMyr was isolated (0.103 mmole, 49%) as a waxy solid. A slow moving band on plates (Rf: 0.10 (Et_2O-hexanes (3:1)) accounted for the rest of the nucleosidic material present (∿ 51%). This reaction product was silylated according to Ogilvie's procedure[173] and was then hydrolyzed with a solution of concentrated ammonium hydroxide in pyridine (1:1) at ambient temperature for 15 hr. TLC showed the presence of 3'-O-TBDMS thymidine suggesting that the acylation side product was 5'-O-myristoyl thymidine. A test reaction indicated that the minimum time required for a

complete reaction was 12 hr. However, using the same stoichiometry but with refluxing for at least 4 hr, the yield of MyrTMyr increased to 83% (116 mg, 0.175 mmole), ∿ 10% of 5'-O-myristoyl thymidine was formed as well as 3'-O-myristoyl thymidine (< 5%) which was characterized by mass spectrometry (m/e = 452). Chromatographic and spectral properties of these derivatives are listed in Table XII.

N^3-(fluoromethyl) thymidine (CXVIIa) and di(thymidin-N^3-yl) Methane (CXVIIIa)

216 mg of mmtT (0.42 mmole) was dissolved in 10 ml of dry dichloromethane (CH_2Cl_2). 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at room temperature for 4 hr. Solvent was then removed under low pressure and the material was dissolved in $CHCl_3$ (20 ml). The solution was extracted with water (4 x 10 ml). The organic layer was collected and evaporated under reduced pressure. The reaction mixture was purified on silica gel plates which were developed twice with Et_2O. 19 mg of the fluoromethyl derivative CXVIIb was obtained (0.035 mmole, 8%). 199 mg of CXVIIIb was obtained (0.19 mmole, 91%) as a resinous solid. Detritylation of the reaction products was done by treatment with 80% aqueous acetic acid at 85°C for 15-30 min. After purification on silica gel plates, CXVIIa was characterized by CMR. Chemical shifts are reported in Table V. Chromatographic

Table XII. Thin-Layer and Ultraviolet Properties of Acylated Compounds

Compound	Rf Values			$\lambda^{EtOH\ 95\%}_{max}$ (nm)	$\lambda^{EtOH\ 95\%}_{min}$ (nm)
*SiTSi (CXIII)	0.38[a]	0.65[b]	0.82[c]	266	233
AcTAc (CXIVa)	0.03[a]	0.09[b]	0.29[c]	265	233
PvTPv (CXIVb)	0.15[a]	0.38[b]	0.65[c]	265	233
MyrTMyr (CXIVd)	0.23[a]	0.54[b]	0.80[c]	265	234
BzTBz (CXIVc)	0.08[a]	0.28[b]	0.53[c]	266	251
$BzdC^{Bz}Bz$	0.22[c]	0.43[d]	0.76[e]	304,261	290,248
$BzdA^{Bz}Bz$	0.13[c]	0.40[d]	0.73[e]	279	254
BzdABz	0.05[c]	0.10[d]	0.34[e]	263	253
*$BzdG^{Bz}Bz$	0.29[c]	0.75[d]	0.84[e]	284,267, 233	---
BzdGBz	0.31[f] (streaks)	0.24[g]	0.64[h]	273(sh), 255,233	---
*dG^{Bz}	0.24[f] (streaks)	0.08[g]	0.26[h]	284,267, 233	---
mmtTSi (LXVc)	0.17[a]	0.47[b]	0.72[c]	266	247
mmtTAc (CXa)	0.05[a]	0.22[b]	0.49[c]	266	251
mmtTPv (CXb)	0.12[a]	0.40[b]	0.67[c]	265	249
mmtTBz (CXc)	0.08[a]	0.33[b]	0.61[c]	265	251
mmtTMyr (CXd)	0.16[a]	0.47[b]	0.71[c]	265	248
trU^{tr}_{Pv} (CXII)	0.31[a]	0.56[b]	0.84[c]	260	244
*TSi	0.05[a]	0.21[b]	0.50[c]	266	233
*SiT	0.02[a]	0.11[b]	0.35[c]	266	234
**TAc	0.00[a]	0.09[b]	0.28[c]	265	233
**TPv	0.02[a]	0.16[b]	0.43[c]	265	233
*PvT	0.01[a]	0.05[b]	0.30[c]	266	233
**TBz	0.02[a]	0.14[b]	0.38[c]	265	247
**TMyr	0.05[a]	0.21[b]	0.50[c]	266	235
MyrT	0.02[a]	0.10[b]	0.28[c]	266	235

Solvent system: a = Et_2O-hexanes (1:1); b = Et_2O-hexanes (3:1); c = Et_2O; d = EtOAc; e = EtOAc-THF (1:1); f = THF; g = $CHCl_3$-MeOH (9:1); h = $CHCl_3$-MeOH (8:2)

*Added for comparison.

**Obtained from acidic hydrolysis of their p-monomethoxytrityl derivative.

and spectral properties of CXVIIa,b and CXVIIIa,b are reported in Table XIII. The mass spectrum of the bis-TBDMS derivative of CXVIIa gave a peak at m/e = 445 corresponding to the $(M-C_4H_9)^+$ ion.

Mol. wt. calcd. for $C_{19}H_{34}N_2O_5FSi_2$: 445.1991
Found: 445.1990

CXVIIIa was also characterized by CMR (Table V). Mass spectrum of the tetra-TMS derivative of CXVIIIa displayed a parent peak at m/e = 784.

Mol. wt. calcd. for $C_{33}H_{60}N_4O_{10}Si_4$: 784.3387
Found: 784.3390

Di-(Deoxyuridin-N^3-yl) Methane (CXXI)

500 mg of deoxyuridine (2.19 mmole) was suspended in 5 ml of CH_2Cl_2. 25 ml of 0.68 M TBAF (17 mmole) was added and the solution was stirred at room temperature for 24 hr. The solvent was removed under reduced pressure and the residue was dissolved in 6 ml of dry pyridine and 6 ml of distilled acetic anhydride. The solution was then stirred at ambient temperature for 3 hr. The reaction mixture was then evaporated under low pressure. The residue was dissolved in $CHCl_3$ (50 ml) and the solution was extracted with water

(4 x 20 ml). The organic layer was collected and evaporated under reduced pressure. The reaction products were then treated with a solution of concentrated ammonium-hydroxide in pyridine (1:1) (∿ 25 ml) for 15 hr at room temperature. After removal of the solvent under low pressure the residue was applied on silica gel plates which were developed with EtOAc-THF (1:1). 81 mg of N^3-fluoromethyl deoxyuridine was isolated as a gum (0.31 mmole, 14%). Plates were further developed with $CHCl_3$-MeOH (7:3) twice affording 429 mg of CXXI (0.92 mmole, 84%) which was characterized by CMR (Table V). Chromatographic and ultraviolet properties of CXXI are given in Table XIII. Mass spectrum of the tetra-TMS derivative of CXXI exhibited a parent peak at m/e = 756.

Mol. wt. calcd. for $C_{31}H_{56}N_4O_{10}Si_4$: 756.307

Found: 756.308

(Deoxyuridin-N^3-yl)-(Thymidin-N^3-yl) Methane (CXXIIa)

563 mg of mmtT (1.1 mmole) and 250 mg of deoxyuridine (1.1 mmole) were suspended in 5 ml of dry CH_2Cl_2. 25 ml of 0.68 M TBAF (17 mmole) was added and the solution was stirred at ambient temperature for 22 hr. After usual work-up, the reaction mixture was purified on silica gel plates which were developed with Et_2O and then with EtOAc. 286 mg of CXVIIIb was isolated (0.27 mmole, 25%). Plates were further developed

with EtOAc-THF (1:1) twice affording 220 mg of CXXIIb (0.29 mmole, 27%). CXXI was probably formed in similar yield but was lost in the aqueous phase. CXXIIb was treated with 80% aqueous acetic acid at 85°C for 15-30 min. After purification on silica gel plates, CXXIIa was submitted to CMR analysis (Table V). Chromatographic and spectral properties of CXXIIa,b are reported in Table XIII. Mass spectrum of the tetra-TMS derivative of CXXIIa gave a parent peak at m/e = 770.

Mol. wt. calcd. for $C_{32}H_{58}N_4O_{10}Si_4$: 770.323

Found: 770.322

N^3-(2-Chloroethyl) Thymidine (CXXIIIb)

101 mg of thymidine (0.42 mmole) was suspended in 10 ml of 1,2-dichloroethane. 6.2 ml of 0.68 M TBAF (4.2 mmole) was added and the solution was stirred at room temperature for 17 hr. The solvent was then removed under reduced pressure and the material was applied to a dry silica gel column (5 cm x 5 cm) which was eluted with acetone. The solvent was then evaporated under low pressure and the residue was applied on silica gel plates which were developed with Et_2O and then with EtOAc-THF (1:1). 85 mg of CXXIIIb was isolated (0.279 mmole, 66%). Table V gives the chemical shifts of the CMR spectrum of CXXIIIb. Table XIII displayed the chromatographic and ultraviolet properties of CXXIIIb.

The mass spectrum of its bis-TMS derivative gave a parent peak at m/e = 448.

Mol. wt. calcd. for $C_{18}H_{33}N_2O_5Si_2Cl$: 448.162

Found: 448.161

1,2-di-(Thymidin-N^3-yl) Ethane (CXXIVb)

216 mg of mmtT (0.42 mmole) was dissolved in 10 ml of 1,2-dichloroethane. 6.2 ml of 0.68 M TBAF (4.2 mmole) was added and the solution was stirred at ambient temperature for 4 hr. The solvent was then evaporated under reduced pressure. After a typical work-up, the reaction mixture was applied on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. 235 mg of CXXIIIa was obtained (0.41 mmole, 97%). Traces of mmtT were also observed (∿ 2-3%). Table XIII showed the chromatographic and spectral properties of CXXIIIa. 143 mg of mmtT^{CH_2CH_2Cl} (CXXIIIa, 0.25 mmole) and 300 mg of thymidine (1.24 mmole) were mixed with 3.7 ml of 0.68 M TBAF (2.48 mmole). The mixture was then evaporated to dryness under low pressure. The residue was dissolved in 1 ml of dry DMF and the solution was stirred at ambient temperature for 62 hr. After usual extractive work-up, the reaction products were purified on silica gel plates which were developed with Et_2O. 97 mg of starting material CXXIIIa was isolated (0.169 mmole, 68%). Plates were further developed

with ethyl acetate and 14 mg of an unidentified tritylated compound was isolated. Plates were then developed with EtOAc-THF (1:1) affording 53 mg of the desired bridged nucleoside CXXIVa (0.068 mmole, 27%). After deprotection with 80% aqueous acetic acid at 85°C for 15-30 min and further purification on silica gel plates, CXXIVb was submitted to CMR analysis (Table V). Chromatographic and ultraviolet properties of CXXIVa,b are listed in Table XIII. The mass spectrum of the tetra-TMS derivative of CXXIVb showed a parent peak at m/e = 798.

Mol. wt. calcd. for $C_{34}H_{62}N_4O_{10}Si_4$: 798.354

Found: 798.357

N^3-(6-Bromohexyl) Thymidine ($T^{(CH_2)_6Br}$, CXXIIId)

727 mg of thymidine (3 mmole) was suspended in a mixture of 10 ml of dry pyridine and 10 ml of 1,6-dibromohexane. 49 ml of 0.68 M TBAF (30 mmole) was added and the solution was stirred at room temperature for 17 hr. The excess THF and pyridine was removed under low pressure and the residue was applied to a dry silica gel column (7 cm x 9 cm) which was eluted first with hexanes and then with Et_2O in order to remove excess 1,6-dibromohexane. The column was further washed with THF. The THF solution was evaporated under reduced pressure and the reaction product was purified on silica gel plates which were developed with Et_2O and then with EtOAc-THF

(1:1). 780 mg of $T^{(CH_2)_6Br}$ was isolated (1.92 mmole, 64%). The reaction product was characterized by CMR (Table V). Table XIII displayed ultraviolet and chromatographic properties of $T^{(CH_2)_6Br}$. Mass spectrum of $T^{(CH_2)_6Br}$ showed a parent peak at m/e = 405.

1,6-di-(Thymidin-N^3-yl) Hexane (CXXIVd)

216 mg of mmtT (0.42 mmole) was suspended in 10 ml of 1,6-dichlorohexane. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at ambient temperature for 6 hr. The solution was then worked up as usual and the reaction mixture was purified on silica gel plates which were developed with Et_2O-hexanes (3:1). 274 mg of CXXIIIc was isolated (0.42 mmole, 100%). Table XIII showed chromatographic and spectral properties of CXXIIIc. 274 mg of $mmtT^{(CH_2)_6Cl}$ (0.42 mmole) and 508 mg of thymidine (2.1 mmole) were stirred with 6.2 ml of 0.68 M TBAF (4.2 mmole) at ambient temperature for 15 hr. After a typical work-up, the reaction mixture was purified on silica gel plates which were developed with Et_2O-hexanes (3:1) and then with Et_2O. 157 mg of starting material CXXIIIc was obtained (0.25 mmole, 59%). Plates were further developed with EtOAc-THF (1:1) affording 119 mg of CXXIVc (0.14 mmole, 34%). After usual detritylation and purification on silica gel plates, $T^{(CH_2)_6T}$ (CXXIVd) was characterized by CMR (Table V). Chromatographic and ultra-

Table XIII. Chromatographic and Ultraviolet Properties of N^3-Haloalkyl and "Bridged" Nucleosides.

Compound	Rf Values: Thin-Layer			Rf Values: Paper B'	Rf Values: Paper A	$\lambda_{max}^{EtOH\ 95\%}$ (nm)	$\lambda_{min}^{EtOH\ 95\%}$ (nm)	$\lambda_{max}^{H_2O}$ (εmax)	$\lambda_{min}^{H_2O}$ (εmin)
mmtT^{CH_2F} (CXVIIb)	0.08[a]	0.21[b]	0.57[c]	--	--	271	250	--	--
mmtT$^{(CH_2)_2Cl}$ (CXXIIIa)	0.08[a]	0.23[b]	0.58[c]	--	--	269	250	--	--
mmtT$^{(CH_2)_6Cl}$ (CXXIIIc)	0.08[a]	0.24[b]	0.60[c]	--	--	268	250	--	--
mmtT^{CH_2}Tmmt (CXVIIIb)	0.10[c]	0.51[d]	0.56[e]	--	--	268	251	--	--
mmtT^{CH_2}dU (CXXIIb)	0.03[e]	0.25[f]	0.56[g]	--	--	266	246	--	--
mmtT$^{(CH_2)_2}$T (CXXIVa)	0.05[e]	0.49[f]	0.74[g]	--	--	267	247	--	--
mmtT$^{(CH_2)_6}$T (CXXIVc)	0.11[e]	0.49[f]	0.76[g]	--	--	267	247	--	--
*T	--	--	--	0.56	0.69	266	234	--	--
*T^{Me}	--	--	--	0.75	0.87	266	236	--	--
T^{CH_2F} (CXVIIa)	0.21[e]	0.56[f]	0.73[g]	0.76	0.85	271	236	271 (7,700)	236 (1,470)
T$^{(CH_2)_2Cl}$ (CXXIIIb)	0.23[e]	0.56[f]	0.71[g]	0.80	0.86	269	236	268 (8,050)	237 (2,210)
T$^{(CH_2)_6Br}$ (CXXIIId)	0.27[e]	0.57[f]	0.65[g]	0.88	0.88	267	236	267 (7,000)	237 (2,310)
T^{CH_2}T (CXVIIIa)	0.11[f]	0.39[g]	0.28[h]	0.53	0.76	268	236	268 (14,600)	237 (3,840)
T^{CH_2}dU (CXXIIa)	0.24[g]	0.19[h]	0.60[i]	0.43	0.74	265	234	265 (15,500)	235 (4,320)
dU^{CH_2}dU (CXXI)	0.15[g]	0.18[h]	0.53[i]	0.32	0.69	263	234	263 (15,300)	234 (3,990)
T$^{(CH_2)_2}$T (CXXIVb)	0.10[f]	0.40[g]	0.32[h]	0.53	0.76	268	236	267 (13,200)	237 (4,140)
T$^{(CH_2)_6}$T (CXXIVd)	0.17[f]	0.44[g]	0.42[h]	0.75	0.83	267	236	267 (15,300)	237 (4,840)

*Added for comparison.

Solvent system: a = Et_2O-hexanes (1:1); b = Et_2O-hexanes (3:1); c = Et_2O; d = Et_2O-EtOAc (1:1); e = EtOAc; f = EtOAc-THF (1:1); g = THF; h = $CHCl_3$-MeOH (8:2); i = $CHCl_3$-MeOH (6:4).

violet properties of CXXIVc,d are given in Table XIII. Mass spectrum of the tetra-TMS derivative of CXXIVd displayed a parent peak at m/e = 854.

Mol. wt. calcd. for $C_{38}H_{70}N_4O_{10}Si_4$: 854.417

Found: 854.418

bis-Butyl Ester of N^3-Butyl-5'-O-Trityl Thymidine-3'-Phosphate (CXXVIa)

126 mg of the diammonium salt of 5'-O-trityl thymidine-3'-phosphate (CXXVa, 0.21 mmole) was suspended in 5 ml of dry 1-chlorobutane. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at 85°C for 24 hr. The solvent was then evaporated at low pressure and the material was dissolved in $CHCl_3$ (10 ml). The solution was extracted with water (4 x 5 ml). The organic phase was collected and concentrated at reduced pressure. The residue was applied on silica gel plates which were developed with Et_2O-hexanes (3:1).

The moving band was eluted with EtOAc while the stationary band was eluted with methanol. 77 mg of the triester CXXVIa was obtained (0.105 mmole, 50%) while 82 mg of the diester CXXVIIa was isolated (0.089 mmole, 43%). After usual detritylation CXXVIIb moved as a homogenous, singly charged spot on paper electrophoresis; no trace of a doubly charged material was observed.

The minimum time required for a complete alkylation reaction was about 8 hr.

An identical reaction was carried out on the same scale but at room temperature. 31 mg of CXXVIa was obtained (0.042 mmole, 20%) along with 130 mg of CXXVIIa (0.14 mmole, 67%). Chromatographic, electrophoretic and spectral properties of both mononucleotide triester and diester, are given in Tables XIV and XV.

The Use of 1-Bromobutane as Alkylating Agent: Preparation of the bis-Butyl Ester of N^3-Butyl-5'-O-Trityl Thymidine-3'-Phosphate (CXXVIa)

126 mg of CXXVa (0.21 mmole) was suspended in 5ml of dry 1-bromobutane. 3.1 ml of 0.68 M TBAF (2.1 mmole) was added and the solution was stirred at ambient temperature for 24 hr. After identical work-up and purification procedures as for the previous preparation, 61 mg of CXXVIa was isolated (0.083 mmole, 40%); ∿ 5-10% of the bis-butyl ester of 5'-O-trityl thymidine-3'-phosphate was also observed. Upon heating at 55-60°C, an identical preparation yielded 77 mg of $trT^{Bu}p^{Bu}_{Bu}$ (0.105 mmole, 50%); ∿ 5-10% of $trTp^{Bu}_{Bu}$ was again observed. Eight hours was the minimum time required for a complete reaction.

Butyl Phosphate Ester of N^3-Butyl-5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N^3-Butyl Thymidine (CXXIXa)

88 mg of the dinucleotide CXXVIIIa (0.105 mmole) was suspended in 2.5 ml of dry 1-bromobutane. 1.54 ml of 0.68 M TBAF (1.05 mmole) was added and the solution was stirred at 55-60°C for 2½ hr. After usual work-up, the reaction mixture was purified on silica gel plates which were developed twice with Et_2O-EtOAc (1:1). 31 mg of CXXIXa was obtained (0.031 mmole, 30%) along with 70 mg of the diester CXXXa (0.066 mmole, 63%). Table XIV displayed the chromatographic and ultraviolet properties of CXXIXa. An aqueous solution of CXXXa was converted to its sodium salt by passing through a column of DOWEX 50W-X8 ($Na^{\oplus}$ ionic form) ion-exchange resin. After conventional detritylation and purification on preparative papers which were developed in solvent B', CXXXb was eluted from paper with water and the solution was lyophilized. CXXXb was then spotted on electrophoresis paper giving rise to a homogenous, singly charged material moving at lower E_m than TpT (Table XV). CXXXb (0.1-1 mg) was also submitted to enzyme degradation. Bovine spleen phosphodiesterase completely degraded the substrate to $T^{Bu}p$ and T^{Bu} while snake venom phosphodiesterase degraded $T^{Bu}pT^{Bu}$ completely to T^{Bu} and pT^{Bu}.

Attempted Synthesis of bis-Methyl Phosphate Ester of N^3-Methyl-5'-O-Trityl Thymidine-3'-Phosphate

80 mg of the mononucleotide CXXVa (0.134 mmole) was dissolved in 1.6 ml of trimethyl phosphate and 1.6 ml of dry THF. 2 ml of 0.68 M TBAF (1.34 mmole) was added and the solution was stirred at room temperature for 24 hr. The reaction mixture was worked-up and converted into its detritylated sodium salt as usual. Purification was achieved on preparative papers which were developed with solvent B'. Two bands were eluted with water and the yield of each band was determined spectrophotometrically. 124.8 $O.D._{265}$ units of $T^{Me}p^{Me}_{OH}$ (CXXXIb, 95.6%) and 6.05 $O.D._{267}$ units of Tp and/or $T^{Me}p$ (4.6%) were obtained. Chromatographic, electrophoretic and spectral properties of CXXXIb are reported in Table XV. A test reaction which was monitored by electrophoresis indicated that 14 hr is the minimum time required for a complete reaction. A test reaction showed also on electrophoresis that no reaction (< 5%) occurred under the conditions described above in the absence of TBAF.

Methyl Phosphate Ester of N^3-Methyl-5'-O-Trityl Thymidilyl-(3'→5')-3'-O-Acetyl Thymidine ($trT^{Me}p(Me)TAc$). Chemical Proof for the Structure of CXXXIa

55 mg of the sodium salt of CXXXIa (0.092 mmole) and

39 mg of 3'-O-acetyl thymidine (0.14 mmole) were carefully dried by coevaporation with dry pyridine (4 x 2 ml). 56 mg of TPS (0.184 mmole) was added followed by ∿ 2 ml of dry pyridine. The solution was then stirred at ambient temperature for 36 hr. Water (∿ 0.5 ml) was added and the solution was stirred for a further hr. The solvent was removed under reduced pressure, the residue was dissolved in $CHCl_3$ (10 ml), and the solution was then extracted with water (4 x 3ml). The organic layer was collected and concentrated under low pressure. The residue was applied on silica gel plates which were developed with Et_2O, EtOAc and EtOAc-THF (1:1). 28 mg of the desired dinucleotide triester $trT^{Me}p(Me)TAc$ (CXXXII) (0.33 mmole, 36% yield based on CXXXIa).

28 mg of $trT^{Me}p(Me)TAc$ was dissolved in a mixture of concentrated ammonium hydroxide and pyridine (1:1) (∿ 15 ml). The solution was stirred at room temperature for 15-18 hr. The solvent was removed under low pressure and the residue was detritylated as usual, the reaction mixture was then applied on preparative papers which were developed with solvent A. Only ∿ 20-30% of the dinucleotide triester was fully deprotected. The nucleotidic band was eluted from paper with water. After lyophilization, the nucleotidic material was subjected to enzymatic degradation. Snake venom phosphodiesterase degraded completely $T^{Me}pT$ to T^{Me} and pT suggesting that the original nucleotide had a methyl group at the N^3-position.

N^3-Methyl-5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N^3-Methyl Thymidine (CXXXIVa)

17 mg of CXXVIIIa (0.021 mmole) was dissolved in 0.25 ml of trimethyl phosphate and 0.25 ml of dry THF. 0.31 ml of 0.68 M TBAF (0.21 mmole) was added and the solution was stirred at ambient temperature for 24 hr. After usual extractions and treatment with $Na^{\oplus}$ ion exchange resin, TLC showed no significant amount of material (< 5%) that could move even with EtOAc-THF (1:1) as developing solvent. The reaction mixture was then detritylated and purified on preparative papers which were developed with solvent B'. After elution and lyophilization, the nucleotidic material was treated with enzymes. Snake venom phosphodiesterase completely degraded the substrate to T^{Me} and pT^{Me} while the bovine spleen phosphodiesterase completely degraded $T^{Me}pT^{Me}$ to $T^{Me}p$ and T^{Me}. Chromatographic, electrophoretic and spectral properties of $T^{Me}pT^{Me}$ are reported in Table XV.

Methyl Phosphate Ester of N^3-Methyl-5'-O-p-Monomethoxytrityl Thymidilyl-(3'→5')-N^3-Methyl Thymidine (CXXXVa)

175 mg of CXXVIIIa (0.210 mmole) was suspended in 5 ml of bromomethane. 2.1 ml of 1 M TBAF was added and the solution was stirred at room temperature for 24 hr. The reaction was then quenched with a solution of ethanol-water (1:1)

Table XIV. Thin-Layer Chromatographic and Spectral Properties of Alkylated Nucleotide Triesters

Compound	Rf Values			$\lambda^{EtOH\ 95\%}_{max}$ (nm)	$\lambda^{EtOH\ 95\%}_{min}$ (nm)
$trT^{Me}{}_{p}{}^{Me}_{Me}$	0.10[b]	0.22[c]	0.26[d]	264	243
$T^{Me}{}_{p}{}^{Me}_{Me}$	0.03[d]	0.20[e]	0.39[f]	264	239
$^{Me}_{Me}pU^{Me}$ (CXXXVIIIa)	0.14[e]	0.42[f]	0.61[g]	261	235
$cU^{Me}{}_{p(Me)}$ (CXXXVIIa)	0.10[d]	0.40[e]	0.62[f]	257	232
$trT^{Et}{}_{p}{}^{Et}_{Et}$	0.32[b]	0.50[c]	0.55[d]	265	243
$T^{Et}{}_{p}{}^{Et}_{Et}$	0.10[d]	0.36[e]	0.56[f]	265	236
$^{Et}_{Et}pU^{Et}$ (CXXXVIIIb)	0.33[e]	0.65[f]	0.69[g]	261	236
$cU^{Et}{}_{p(Et)}$ (CXXXVIIb)	0.17[d]	0.54[e]	0.70[f]	258	234
$trT^{Bu}{}_{p}{}^{Bu}_{Bu}$ (CXXVIa)	0.37[a]	0.66[b]	0.81[c]	265	244
$T^{Bu}{}_{p}{}^{Bu}_{Bu}$ (CXXVIb)	0.08[a]	0.26[b]	0.35[c]	266	236
$mmtT^{Me}{}_{p(Me)}T^{Me}Ac$	0.11[c]	0.26[d]	0.71[e]	266	246
$mmtT^{Me}{}_{p(Me)}T^{Me}$ (CXXXVa)	0.03[c]	0.09[d]	0.55[e]	265	246
$T^{Me}{}_{p(Me)}T^{Me}Ac$	0.42[e]	0.64[f]	0.69[g]	264	235
$T^{Me}{}_{p(Me)}T^{Me}$ (CXXXVb)	0.16[e]	0.45[f]	0.63[g]	265	236
$trT^{Et}{}_{p(Et)}T^{Et}Ac$	0.26[c]	0.45[d]	0.83[e]	264	241
$trT^{Et}{}_{p(Et)}T^{Et}$	0.05[c]	0.17[d]	0.68[e]	266	242
$T^{Et}{}_{p(Et)}T^{Et}$	0.27[e]	0.68[f]	0.73[g]	265	236
$mmtT^{Bu}{}_{p(Bu)}T^{Bu}Ac$	0.58[c]	0.64[d]	0.18[b]	266	246
$mmtT^{Bu}{}_{p(Bu)}T^{Bu}$ (CXXIXa)	0.26[c]	0.45[d]	0.69[e]	266	246
$T^{Bu}{}_{p(Bu)}T^{Bu}Ac$	0.18[d]	0.73[e]	0.81[f]	265	236
$T^{Bu}{}_{p(Bu)}T^{Bu}$ (CXXIXb)	0.48[e]	0.72[f]	0.66[g]	266	236

Solvent system: a = Et_2O-hexanes (3:1); b = Et_2O; c = Et_2O-EtOAc (1:1); d = EtOAc; e = EtOAc-THF (1:1); f = THF; g = $CHCl_3$-MeOH (8:2).

Table XV. Paper Chromatographic, Electrophoretic and Spectral Properties of Alkylated Nucleotide Diesters.

Compound	B'	A	E_m*	$\lambda^{H_2O}_{max\,(nm)}$	$\lambda^{H_2O}_{min\,(nm)}$	$\lambda^{pH=1}_{max\,(nm)}$	$\lambda^{pH=1}_{min\,(nm)}$	$\lambda^{pH=13}_{max\,(nm)}$	$\lambda^{pH=13}_{min\,(nm)}$
**$T^{Me}{}_{p}{}^{Me}{}_{O^{\ominus}}$ (CXXXIb)	0.20	0.68	0.59	265	236	264	236	265	236
**$T^{Et}{}_{p}{}^{Et}{}_{O^{\ominus}}$	0.36	0.83	0.57	267	237	267	237	266	237
**$T^{Bu}{}_{p}{}^{Bu}{}_{O^{\ominus}}$ (CXXVIIb)	0.67	0.85	0.45	266	238	266	238	266	238
†TpT (CXXVIIIb)	0.08	0.39	0.44	-	-	-	-	-	-
**$T^{Me}{}_{pT}{}^{Me}$ (CXXXIVb)	0.23	0.72	0.39	265	237	266	237	266	236
**$T^{Et}{}_{pT}{}^{Et}$	0.35	0.86	0.34	266	238	266	237	267	238
**$T^{Bu}{}_{pT}{}^{Bu}$ (CXXXb)	0.57	0.87	0.33	267	238	267	238	268	238

*Electrophoretic mobilities after 1.5 hr in triethylammonium bicarbonate buffer (0.05 M, pH = 7.5)

**Sodium salt.

†Ammonium salt.

(1 ml) and the solvent was removed under low pressure. After a conventional work-up, the reaction mixture was applied on silica gel plates which were developed with EtOAc and then with EtOAc-THF (1:1). Three bands were observed. The band of largest Rf yielded 99 mg of CXXXVa (0.115 mmole, 55%). Table XIV showed the chromatographic and ultraviolet properties of CXXXVa. The middle band gave 18 mg of a tritylated unidentified material. The lowest band which constituted the base line gave after elution with methanol, 97 mg of the diester CXXXIVa (0.089 mmole, 42%). The diester was treated as usual with the $Na^{\oplus}$ ion exchange resin and was then detritylated. After purification on preparative papers developed with solvent B' and after elution and lyophilization, the nucleotidic material was subjected to enzyme degradations. The substrate was completely degraded by snake venom phosphodiesterase to T^{Me} and pT^{Me} while the bovine spleen phosphodiesterase completely degraded $T^{Me}pT^{Me}$ to $T^{Me}p$ and T^{Me}.

General Procedure for Preparative Scale Alkylation Reactions Using Dimethylsulfate or Diethylsulfate as Alkylating Agent

0.21 mmole of the mononucleotide CXXVb or the dinucleotide CXXVIIIb was dissolved in 1.54 ml of 0.68 M TBAF (1.05 mmole). 2.1 mmole of the desired alkylating agent was added and the solution was stirred at room temperature for 30 min. Identical addition of TBAF/alkylating agent was repeated at every 30 min

until the total amount of TBAF/alkylating agent reached 5.25 mmole and 10.5 mmole respectively. After a total time of 3 hr, the solution was carefully concentrated under low pressure, without heating, to a volume of ∿ 2 ml. The residue was poured onto 25 ml of crushed ice (mixed with 5 ml of water). The reaction flask was rinsed with ∿ 1 ml of acetone which was also added to the ice. The cold mixture was then gently stirred with a spatula. Usually, the desired triester precipitated out but when this was not the case an organic layer composed of the desired triester and alkylating agent formed beneath an aqueous layer. The aqueous layer containing TBAF and probably some sulfonic acid, was carefully decanted. The precipitate or the organic layer was dissolved with $CHCl_3$ (20 ml) and the solution was washed once with water (10 ml). The organic phase was concentrated under low pressure and the residue was purified on silica gel plates from which phosphotriesters were isolated in high yields. The combined aqueous phases were concentrated under low pressure and treated with the $Na^{\oplus}$ ion exchange resin. A concentrate solution of the residue coming from the aqueous phase was spotted for TLC, paper chromatography and electrophoresis. None of them showed tritylated or untritylated nucleotides. Therefore, almost all nucleotidic material (> 95%) precipitated out or was in the organic layer at the bottom of the beaker.

General Procedure for Small Scale Alkylation Reactions Using Dialkyl Sulfate or Alkyl Alkanesulfonates as Alkylating Agents

0.021 mmole of the mononucleotide CXXVb or the dinucleotide CXXVIIIb was dissolved in 0.16 ml of 0.68 M TBAF (0.105 mmole). 0.21 mmole of the alkylating agent was added and the solution was stirred at room temperature for 30 min. The combined addition of TBAF and alkylating agent was repeated as described in the previous procedure. After a total time of 3-5 hr the reaction mixture was concentrated under low pressure and the residue was passed through a column of $Na^{\oplus}$ ion exchange resin (DOWEX 50W-X8), at the rate of ∿1 drop/sec. After evaporation of the water under reduced pressure, the residue was applied on preparative papers which were developed with the appropriate solvent (A or B'). Bands were eluted from paper with water and their yields were determined spectrophotometrically. Results are reported in Table VI.

Methyl Ester of N^3-Methyl Uridine-(3'→5') Cyclic Phosphate (CXXXVIIa)

28 mg of the sodium salt of uridine-(3'→5') cyclic monophosphoric acid (CXXXVI, 0.084 mmole) was dissolved in 1.84 ml of 0.68 M TBAF (1.26 mmole). 0.2 ml of dimethylsulfate (2.1 mmole) was added and the solution was stirred at ambient temperature for 3 hr. The reaction mixture was then worked-up as for the previous procedures. After purification on

preparative paper developed with solvent B', three bands were eluted and gave 6.05 $O.D._{259}$ units (6.5%) of an unidentified material moving a little faster than CXXXVIIa, 78.4 $O.D._{258}$ units of the cyclic triester (CXXXVIIa, 83.8%), and 9.1 $O.D._{258}$ units of what is probably N^3-methyl uridine-(3'→5') cyclic phosphate (9.7%). CXXXVIIa was silylated according to Ogilvie's procedure[173] and was submitted to mass spectrometric analysis. An intense peak at m/e = 391 accounted for the $(M-C_4H_9)^+$ ion (Fig. VIII).

The ethyl ester CXXXVIIb was also synthesized, using each alkylating agent. The yields are reported in Table VI. Chromatographic and spectral properties of these compounds are listed in Table XIV.

Reaction of the Sodium Salt of Uridine-5'-Phosphate with Dimethyl Sulfate in the Presence of TBAF (Preparation #1)

This experiment was carried out according to the general procedure for small scale reactions including work-up and purification steps. From the papers developed in solvent A, two bands were eluted and their yields determined spectrophotometrically. 97.9 $O.D._{261}$ units of the triester CXXXVIIIa (94.6%) and 5.60 $O.D._{261}$ units of the diester CXLI (5.4%) were obtained. The ethyl triester and diester derivatives were similarly synthesized and their chromatographic and spectral properties are given in Table XIV.

Reaction of the Pyridinium Salt of Uridine-5'-Diphosphate (CXXXIX) with Dimethyl Sulfate in the Presence of TBAF (Preparation #2)

10 mg of CXXXIX (0.021 mmole) was dissolved in 0.77 ml of 0.68 M TBAF (0.525 mmole). 0.1 ml of dimethylsulfate (1.05 mmole) was added and the solution was stirred at room temperature for 3.25 hr. The mixture was then worked-up as previously. Two bands were eluted with water from preparative papers which were developed in solvent A. The fast moving band gave 51.7 $O.D._{260}$ units (36.4%) of a material which gave a PMR spectrum identical to that given by CXXXVIIIa. The slow band (Rf^{A} = 0.56) gave 90.4 $O.D._{262}$ units (63.6%) of a material giving a PMR spectrum identical to that given by CXLI.

Reaction of the Pyridinium Salt of Uridine-5'-Triphosphate (CXL) with Dimethyl Sulfate in the Presence of TBAF (Preparation #3)

11 mg of CXL (0.021 mmole) was dissolved in 0.77 ml of 0.68 M TBAF (0.525 mmole). 0.1 ml of dimethylsulfate (1.05 mmole) was added and the solution was stirred at ambient temperature for 3.25 hr. The reaction mixture was worked-up and purified on preparative papers (solvent A) as for previous preparations. 30.2 $O.D._{261}$ units (23.1%) of a material having a superimposable PMR spectrum with that of CXXXVIIIa was obtained along with 95.2 $O.D._{261}$ units (72.7%) of a material producing the identical PMR spectrum as CXLI. Furthermore,

chromatographic and spectral properties of all preparations (#1, 2 and 3) seem to be identical for all the reaction products (see below).

Preparation (#)	Rf(EtOAc-THF(1:1))	Rf(THF)	Rf($CHCl_3$-MeOH(8:2))	$\lambda^{EtOH\ 95\%}_{max}$	$\lambda^{EtOH\ 95\%}_{min}$
1 (CXXXVIIIa)	0.14	0.42	0.61	261	235
2	0.16	0.44	0.61	260	233
3	0.16	0.42	0.57	261	234

Preparation (#)	$Rf^{B'}$	Rf^{A}	Rf^{F}	E_m*
1 (CXLI)	0.04	0.56	0.81	0.46
2	0.04	0.56	0.81	0.47
3	0.04	0.56	0.81	0.46

Reaction of CXXXVIIIa with TBAF in dry THF

50 mg of CXXXVIIIa (0.14 mmole) was dissolved in 2.1 ml of 0.68 M TBAF (1.4 mmole). The reaction was monitored by electrophoresis. It appeared that between 60 and 90 min, starting material had completely reacted affording CXLI in nearly quantitative yield. The reaction mixture was treated as usual with the $Na^{\oplus}$ ion exchange resin and was then applied on preparative papers which were developed with solvent A. CXLI was obtained as an homogenous band without any detectable side products

*After 1.5 hr in triethylammonium-bicarbonate buffer (0.05 M, pH 7.5).

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