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INTRA- AND INTERMOLECULAR NUCLEOPHILIC PHOSPHORUS - SULFUR BOND CLEAVAGE. THE REACTION OF FLUORIDE ION WITH O-ARYL-O,S-DIALKYLPHOSPHOROTHIOATES, & THE DEGRADATION OF PHOSPHOROTHIOATE LINKAGE IN *di*-RIBONUCLEOTIDES BY THE VICINAL 2′-HYDROXYL GROUP.

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Summary: *O-aryl-O,S-dialkylphosphorothioates, such as fully-protected adenylyl(3′→5′)-5′-thiouridine* ***11****, O-aryl-O-ethyl-5′-thiouridyl phosphorothioate* ***24****, upon treatment with an excess n-tetrabutylammonium fluoride in tetrahydrofuran-pyridine-water (8:1:1 v/v/v) underwent a scission of phosphorus-sulfur bond to give the corresponding O-alkylphosphoromonofluoridates* ***20*** *(74%), and* ***30*** *(75%). This facile, new preparation of alkylphosphoromonofluoridates has been found to be a general reaction which has been exemplified by the conversion of O-aryl-O,S-dialkylphosphorothioates* ***26, 27, 28*** *and* ***29*** *to the corresponding phosphorofluoridates* ***30*** *(79%), (****31****+* ***32****, together 85%),* ***33*** *(63%), and* ***34*** *(90%) The fully-protected adenylyl(3′→5′)-5′-thiouridines* ***12*** *and* ***13*** *were partially deprotected to* ***37*** *and* ***38****, having a phosphorothioate linkage with a bridging sulfur [ribonucleoside-3′-O-PO_2-S-5′-ribonucleoside] in order to examine the stability of this internucleotidyl linkage vicinal to a 2′-hydroxyl function. The 2′-O-protected adenylyl(3′→5′)-5′-thiouridines* ***37*** *and* ***38*** *were found to be as stable as thymidylyl(3′→5′)-5′-thiothymidine* ***41****. Removal of the 2′-O-protecting group from* ***37*** *or* ***38*** *however gave only a transiently stable adenylyl(3′→5′)-5′-thiouridine* ***39*** *which promptly decomposed through a phosphorus -sulfur bond cleavage, due to the nucleophilc attack by the vicinal 2′-hydroxyl group, both under mildly acidic and neutral conditions, to give the 2′,3′-cyclic phosphate* ***40*** *(~75%), and 5′-thiouridine derivatives* ***18*** *and* ***19****.*

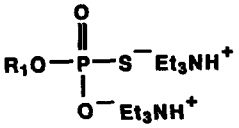
The modification of the phosphate diester linkage in oligonucleotide analogues are of considerable interest because of their potential use as therapeutic or diagnostic reagents[2-4,7], and also as novel tools for studying various biochemical processes[5-7]. The oligonucleotide analogues having modification in the phosphodiester linkage which could be particularly important are the ones which are isosteric and isopolar with the normal phosphodiester. Since these analogues are expected to mimic the conformation of natural DNA, and therefore, may show biochemical properties similar to natural DNA. An additional advantage is that these analogues are nuclease resistant[8,9]. Up till now, the most biochemically useful oligonucleotide analogues with modification at the phosphate are those of DNA analogues containing chiral phosphorothioate linkages[1-7,10]. Caruthers et al. have also recently shown that achiral phosphorodithioate internucleotide linkage might be of improved therapeutic value[8,9] against HIV infections than those with the chiral phosphorothioates[11]. Syntheses of DNA analogues having achiral internucleotide phosphorothioate linkages, in which the bridging oxygen has been replaced by sulfur either at the 3′-position[12] [nucleoside-3′-S-PO_2-O-5′-nucleoside], or at the 5′-position[13-16] [nucleoside-3′-O-PO_2-S-5′-nucleoside], have been reported. Oligoribonucleotides containing the chiral phosphorothioate linkage [nucleoside-3′-O-PO[S]-O-5′-nucleoside] has also been successfully synthesized[17-20], but no work has yet been reported, to the best of our knowledge, on synthesis of oligo*ribo*nucleotides having phosphorothioate linkages with a bridging sulfur such as [*ribo*nucleoside-3′-S-PO_2-O-5′-*ribo*nucleoside], or [*ribo*nucleoside-3′-O-PO_2-S-5′-*ribo*nucleoside]. We therefore became interested in the investigation of their synthesis which would settle the question if such analogues of oligoribonucleotide having achiral

phosphorothioates with bridging sulfur are at all stable in their deprotected form, and if so these RNA analogues would prove to be useful tools in many biochemical applications including RNA catalysis[21] and splicing[21].

It has been shown that the internucleotide phosphotriester linkage in oligoribonucleotides is cleaved by the vicinal 2′-hydroxyl function under mild acidic conditions[22] and is also very labile under neutral conditions[23,24]. This is also found to be true for oligoribonucleotides having phosphorothioate linkages formed upon alkylation of the phosphorothioate linkage[25]. On the other hand, the phosphodiester linkage of the natural oligoribonucleotide is stable under neutral conditions and reasonably stable under acidic condtions[26], but it does not appear to be self-evident that a corresponding *oligoribonucleotide analogue having phosphorothioate linkage with the bridging sulfur* would have a comparable stability under an identical condition. A perusal of the pK_a′s of ethanol and ethanethiol (15.5 and 10.5, respectively) suggests that, since ethanethiol is more acidic by five orders of magnitude, the mercaptide ion is more stable in aqueous media than the primary alkoxide ion. This suggests that the P-S bond in various phosphorothioates such as [nucleoside-3′-O-PO_2-S-5′-nucleoside] is more susceptible to cleavage under hydrolytic conditions than the P-O bond in natural phosphodiesters. Clearly, this should be even more favourable in the oligoribonucleotide analogue having phosphorothioate linkage with the bridging sulfur due to the influence of the vicinal hydroxy group. In order to test this rational, we chose to synthesize fully protected [*ribo*nucleoside-3′-O-PO_2-S-5′-*ribo*nucleoside] with a bridging sulfur at the 5′-position, since it involves the preparation of much simpler building blocks than those required for the synthesis of [*ribo*nucleoside-3′-S-PO_2-O-5′-*ribo*nucleoside].

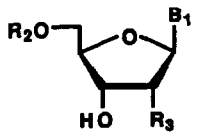
***Synthesis of protected ribonucleoside-3′-O-PO_2-S-5′-ribonucleoside* 11 - 13.** The synthetic strategy developed by Nagyvary and co-workers[13-15] is based upon a nucleophilic displacement of the 5′-O-tosylate group in an appropriately protected nucleoside by the 3′-O-phosphorothioate monoester salt of a nucleoside block . This method had the typical disadvantages associated with the nucleic acid synthesis using "the phosphodiester approach" [27]. We have therefore devised new thiophosphorylating reagents such as **1** and **2**, containing phosphate-protecting groups which are commonly used in nucleotide chemistry using the "phosphotriester approach" [28]. Our synthetic strategy for the target protected dimers **11** - **13** is outlined in Scheme 1. By hydrolysing O-(2-chlorophenyl)phosphorodichloridothioate[29], using a modified literature procedure[30], bis-triethylammonium salt of 2-chlorophenylphosphorothioate (**1**) [$\delta^{31}P$ = +50.45] was obtained in 63% yield. Similarly, bis-triethylammonium salt of 2-cyanoethylphosphorothioate (**2**) [$\delta^{31}P$ = +56.25] was obtained in 36% yield using a procedure described for the corresponding oxygen analog[31]. The 5′-S-phosphorothioates **8** [$\delta^{31}P$ = +12.46], **9** [$\delta^{31}P$ = +15.94], and **10** [$\delta^{31}P$ = +15.69] were then synthesized in 46, 68 and 58% yield, respectively, by treating the 5′-O-mesylnucleosides **6** and **7** with the thiophosphorylating reagents **1** and **2**, under similar conditions as reported by Nagyvary and co-workers[14]. In the next step **8**, **9** and **10** were condensed with the appropriately protected 3′-hydroxy blocks **3**, **4** and **5**, using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT)[32] as condensing agent under standard reaction conditions commonly employed in the triester approach[28]. This gave the protected (3′ → 5′) diribonucleoside having 5′-S-phosphorothioates **11** [$\delta^{31}P$ = +23.4 & +24.3], **12** [$\delta^{31}P$ = +26.6 & +27.3] and **13** ($\delta^{31}P$ = +28.34 & +28.44] in 73, 33 and 52% yield, respectively. An extension of the same approach [**2** + **7** → **10**, and **10** + **5** → **14**] gave also the protected dithymidine-5′-S-phosphorothioate **14** [$\delta^{31}P$ = +27.71], which was obtained in 63% yield. Thus, protected dinucleoside-5′-S-phosphorothioates can be made in a versatile manner by this approach, employing easily accessible and stable starting materials and intermediates.

Scheme 1 : Synthesis of fully protected dimers 11 - 14



1 : R_1 = Ar

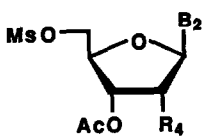
2 : R_1 = $NCCH_2CH_2$



3 : B_1 = A^{Bz}, R_2 = Tol, R_3 = O-Px

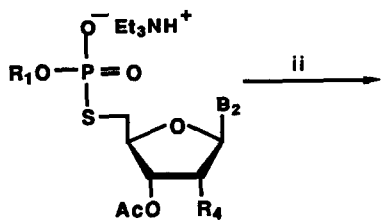
4 : B_1 = A^{Bz}, R_2 = Tol, R_3 = O-TBDMS

5 : B_1 = T, R_2 = MMTr, R_3 = H



6 : B_2 = U^{Bz}, R_4 = OAc

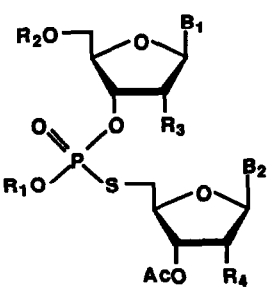
7 : B_2 = T^{Bz}, R_4 = H



8 : B_2 = U^{Bz}, R_1 = Ar, R_4 = OAc

9 : B_2 = U^{Bz}, R_1 = $NCCH_2CH_2$, R_4 = OAc

10 : B_2 = T^{Bz}, R_1 = $NCCH_2CH_2$, R_4 = H



11 : B_1 = A^{Bz}, B_2 = U^{Bz}, R_1 = Ar, R_2 = Tol, R_3 = O-Px, R_4 = OAc

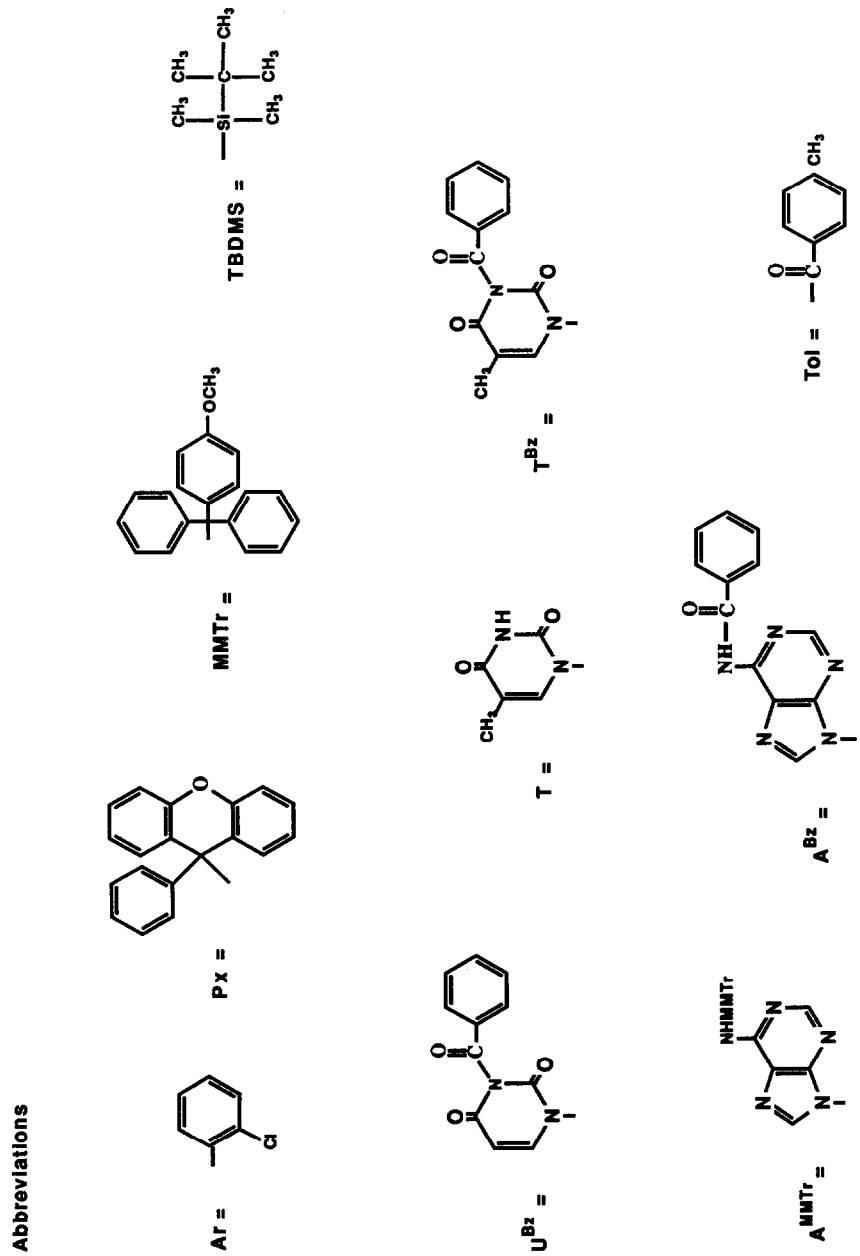
12 : B_1 = A^{Bz}, B_2 = U^{Bz}, R_1 = $NCCH_2CH_2$, R_2 = Tol, R_3 = O-Px, R_4 = OAc

13 : B_1 = A^{Bz}, B_2 = U^{Bz}, R_1 = $NCCH_2CH_2$, R_2 = Tol, R_3 = O-TBDMS, R_4 = OAc

14 : B_1 = T, B_2 = T^{Bz}, R_1 = $NCCH_2CH_2$, R_2 = MMTr, R_3, R_4 = H

(i) (1) or (2) (0.5 eq.), DMF, 3-4 days at 20 °C ; (ii) (3),(4) or (5) (0.91 eq.), MS-NT, (5 eq.) pyridine, 4 h at 20 °C

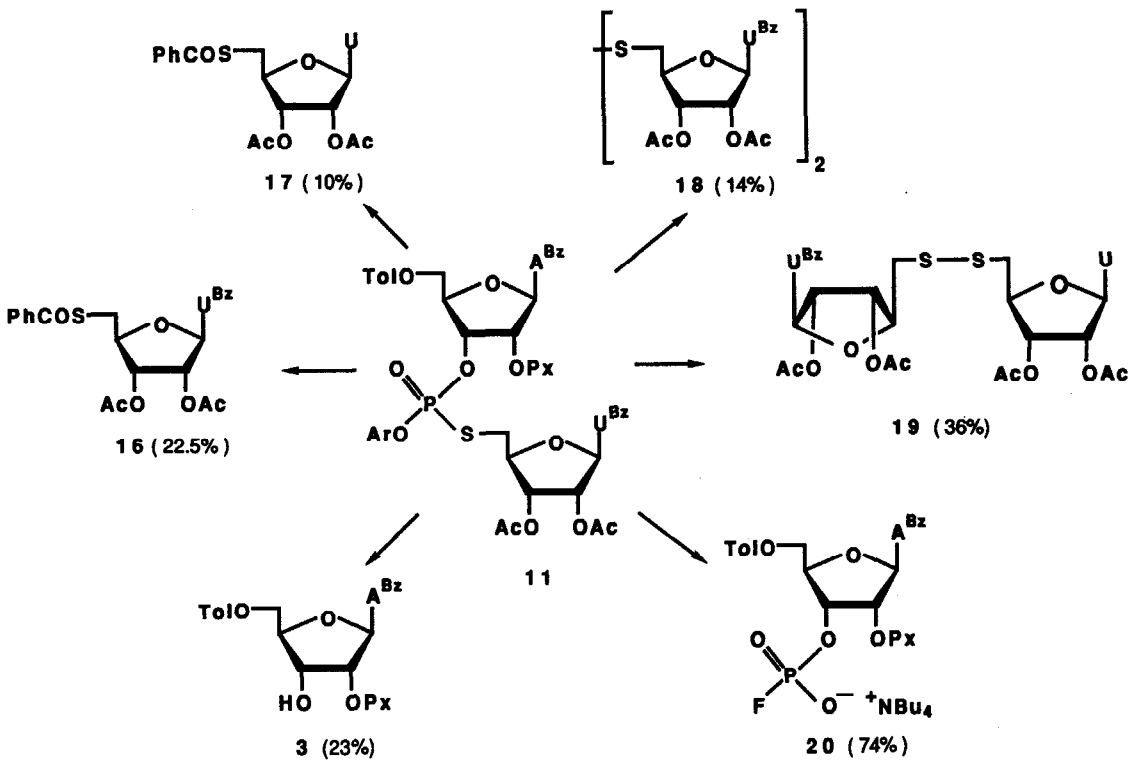
Abbreviations



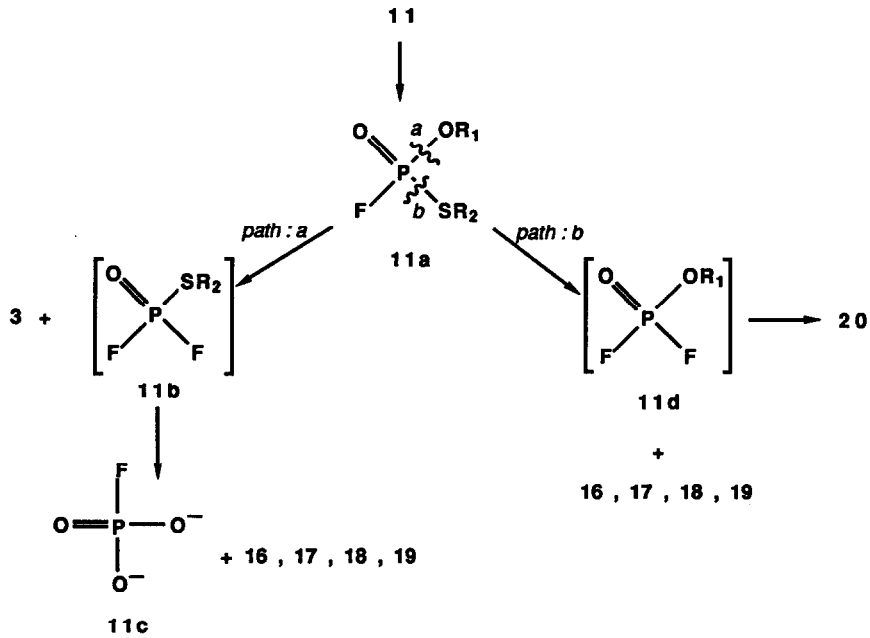
Reaction of protected ribonucleoside-3′-O-PO$_2$-S-5′-ribonucleoside 11 with fluoride ion. We subsequently undertook a study of the chemical behavior of the 5′-S-phosphorothioate linkage in the fully protected diribonucleoside phosphorothioate **11** during the removal of the *o*-chlorophenyl group by action of n-tetrabutylammonium fluoride (TBAF) in THF-pyridine-water (8:1:1 v/v/v)[33]. The result is outlined in Scheme 2. The major product formed in this reaction was n-tetrabutylammonium 6-N-benzoyl-2′-O-pixyl-5′-O-toluoyladenosine-3′-phosphoromonofluoridate (**20**) [δ^{31}P = +5.62, and -20.22; *d*, J_{PF} = 937 Hz], isolated in 74% yield, together with 6-N-benzoyl-2′-O-pixyl-5′-O-toluoyladenosine (**3**) isolated in 22.5% yield. The other products which have been isolated and characterized in this reaction are **16** (23%), **17** (10%), **18** (14%), and **19** (36%), which are secondary products formed from the transient 5′-deoxy-5′-mercapto-2′,3′-di-O-acetyl-3-N-benzoyluridine. Several workers in the early sixties[34,35] managed to show that 5′-thiopentofuranosyl pyrimidines undergoes various rections in a pH-dependent manner due to the 5′-mercapto group. Goodman and co-workers[35] showed that 5′-thiouridine at pH 7 exists in an equilibrium with 5′-deoxy-5′,6-epithio-5,6-dihydrouridine. At pH 13, the 5′-thiouridine dimerisized to its corresponding disulfide[35]. It should be noted that we were unable to observe any cyclic 5′,6-S-anhydrouridine formed in our reaction mixture. Another observation was that the N^3-benzoyl group of uridine was partially deblocked under these conditions and gave raise to a more complicated product mixture such as the disulfides **18** and **19**. Formation of 5′-S-benzoylated uridines **16** and **17** takes place either by a direct intermolecular nucleophilic displacement reaction by the 5′-mercaptide ion on the N^3-benzoyl group of uridine moiety, or by benzoyl fluoride formed from the debenzoylation N^3-benzoyl group of uridine moiety by fluoride ion. Since the reaction of an excess n-tetrabutylammonium fluoride with 2′,3′-di-O-acetyl-N^3-benzoyl uridine deblocks the N^3-benzoyl group within 1 h at 20 °C to a large extent, it is therefore likely that the fluoride ion gave the debenzoylated product **19** from **18**, and benzoyl fluoride formed thereof is presumably the acylating agent giving products **16** and **17**.

The reaction mechanism explaining the formation of various products in the fluoride-promoted degradation of dimer **11** is shown in the Scheme 3. The displacement of the *o*-chlorophenyl group in **11** by fluoride ion gives the unstable diribonucleoside having a 5′-thiophosphoromonofluoridate linkage, as in **11a**, in analogy with the reaction of fluoride ion with the ordinary phosphotriester linkage[36]. However, in this particular case, a second fluoride ion attacks which may orchestrate the cleavage of either the P-OR$_1$ bond (path *a*) or the P-SR$_2$ bond (path *b*). Examination of the composition of products reveals that path *b* takes place in precedence over path *a* in an approximate 3:1 ratio. This finding is consistant with the stabilities of alkoxide versus alkanethiolate ions in the aqueous solutions, reflecting the stability of the -P-SR$_2$ bond versus the -P-OR$_1$ bond. Path *b* gives the intermediary 3′-phosphorodifluoridate **11d**, through the cleavage of -P-SR$_2$ bond, which immediately undergoes hydrolysis to give the isolated 3′-phosphoromonofluoridate **20** (74%) Path *a* suggests formation of a 5′-deoxyuridine-5′-S-thiophosphorodifluoridate block such as **11b** through the cleavage of -P-OR$_1$ bond which was not however detectable in the reaction mixture suggesting that it might have been decomposed to various uridine derivatives **16**, **17**, **18**, **19** and a phosphate component. An examination of ^{31}P-NMR spectrum of the crude reaction mixture indeed showed the presence of the expected monofluorophosphoric acid **11c** as a minor component [δ ^{31}P = +11.3, and -15.0, *d*, J_{PF} = 955 Hz][37,38], thus corroborating the minor fragmentation route of thiophosphoromonofluoridate **11a** through path *a* in Scheme 3.

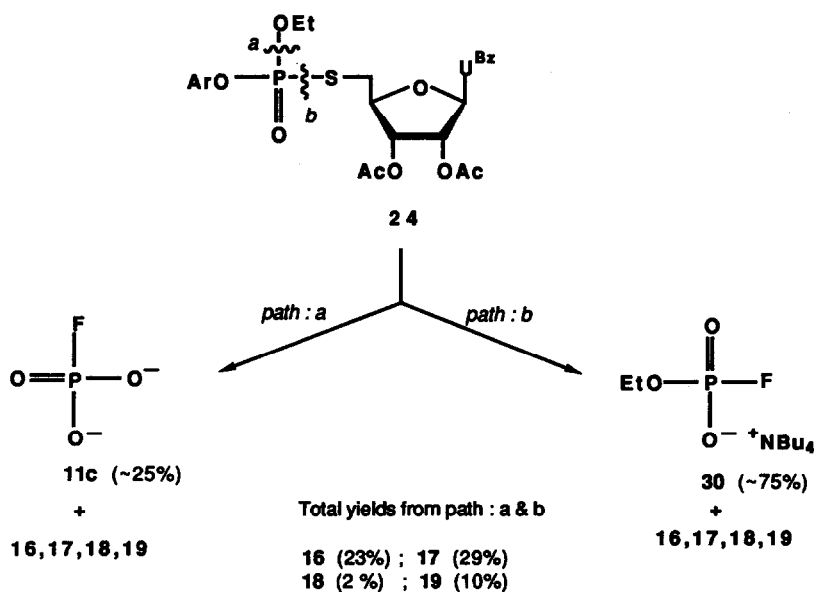
Scheme 2 : Products formed from the reaction of dimer **11** with n-tetrabutylammonium fluoride, (2 eq.) in *THF - pyridine - water (8:1:1 v/v/v) , 1 h at 20 ° C*



Scheme 3 : Proposed pathways for degradation of dimer **11** by action of n-tetrabutylammonium fluoride (2 eq.) in *THF - pyridine - water (8:1:1 v/v/v), 1 h at 20 ° C*



Scheme 4 : Degradation of O-(2-chlorophenyl)-O-ethyl-S-(5′-deoxy-3-N-benzoyl-2′,3′-di-O-acetyl-5′-uridyl)thiophosphate (**24**) by action of n-tetrabutylammonium fluoride (2 eq.) in *THF - pyridine - water (8:1:1 v/v/v), 1 h at 20 ° C*

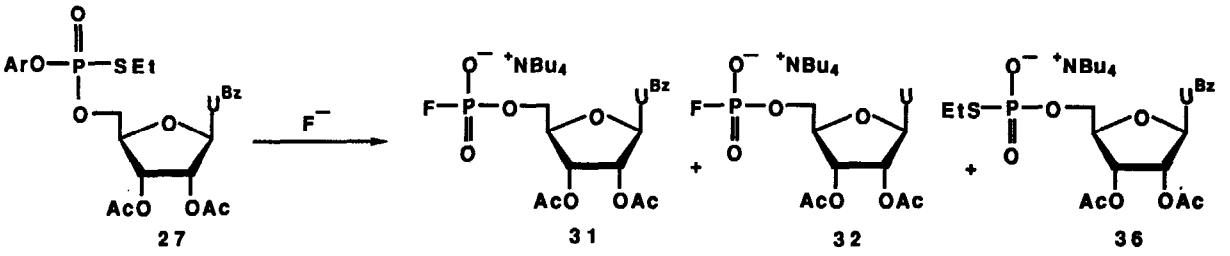


A general route of preparation of phosphoromonofluoridates. As the next stage of this work, the model O,O,S-phosphorothioates **24** and **26** were synthesized in order to get a better picture of the proposed mechanism and also to show the generality of the hitherto unreported preparation of phosphoromonofluoridate starting from O-aryl-O,S-dialkylphosphorothioate. Thus, compound **8** was condensed with ethanol in presence of MSNT, in a similar way as described for the preparation of the fully protected dimer **11**, to give compound **24** [δ ^{31}P = +23.45] in 89% yield. Compound **26** [δ ^{31}P = +25.2] was synthesized in 88% yield according to the general procedure outlined in Scheme 7, by condensing ethanol with compound **25** [δ ^{31}P = +15.67] in presence of MSNT. Compound **25** was obtained in 75% yield by simple S-alkylation of the disodium salt analog of **1** by excess ethylbromide in dimethylformamide. When compound **24** was subjected to the treatment of fluoride ions under an identical condition as described for dimer **11**, a similar degradation pattern was observed (*Scheme 4*). *After an aqueous work up, the O-ethyl phosphoromonofluoridate* **30** *was clearly* identified by its characteristic ^{31}P-NMR [δ ^{31}P = +6.41, and -18.99 ; *d*, J_{PF} = 922 Hz] and ^{1}H-NMR spectrum [dq, δ ^{1}H = 4.0, $^{3}J_{HCOP}$ = 7.6 Hz, and $^{4}J_{HCOPF}$ = 2.4 Hz] along with monofluorophosphoric acid[37,38] **11c** [δ ^{31}P = +12.22, and -14.11 ; J_{PF} = 955 Hz] in 3 : 1 ratio in the crude reaction mixture. Compounds **11c** and **30** could not be isolated pure. 5′-Thiouridine derivatives **16** (23%), **17** (29%), **18** (2%), and **19** (10%) were however isolated along with a few minor unidentified compounds. O-(2-chlorophenyl)-O,S-diethylphosphorothioate **26** was treated with fluoride ion and worked up (Scheme 5) in an identical manner as *described for* **11** *and* **24**. *A* ^{31}P- *and* ^{1}H-NMR *spectra of the crude residue obtained from concentration of the* aqueous phase showed the presence of O-ethylphosphorofluoridate **30**, monofluorophosphoric acid **11c** [δ ^{31}P = +12.22, and -14.17 ; *d*, J_{PF} = 956 Hz], and O,S-diethylphosphorothioate **35** [δ^{31}P = +15.82 ppm] in 79 : 11 : 10 ratio which was calculated on the basis of the integration of the ^{31}P signals. Compound **35** is presumably have formed from direct aqueous hydrolysis of an intermediary O,S-diethyl thiophosphoromonofluoridate. It should be noted on the other hand that O,S-diethylphosphorothioate **35** (80%) was the sole product formed when **26** was hydrolysed either in a mixture of triethylamine-water-dioxane or with triethylammonium fluoride in an *aqueous* THF-pyridine mixture.

Due to these observations, a more systematic study was performed on how the change of stoicheometry of fluoride ion versus water in the reaction mixture can influence the formation of various products due to attack by fluoride or hydroxide ion on O-(2-chlorophenyl)-O-(3-N-benzoyl-2′,3′-O-di-acetyl-5′-uridyl)-S-ethyl phosphorothioate **27** (Table 1). Compound **27** was thus synthesized [δ ^{31}P = +26.61 & +27.15] in 82% yield according to the general synthetic procedure outlined in Scheme 7. It was then subjected to various amounts of TBAF in different aqueous mixtures of tetrahydrofuran and pyridine for a period of 30 min at 20 °C (Table 1). This shows the expected tendency that a large excess of fluoride ion reduces the formation of O,S-phosphorothioate **36** [δ ^{31}P = +17.0, compare expt.#1 with #3 in Table 1], and increases the yield of **32** [δ^{31}P = +6.5 & -19.11; *d*, J_{PF} = 929 Hz, compare expt.#1 with #3 and 4 in Table 1] due to N^{3}-debenzoylation of **31** [δ ^{31}P = +6.32 & -19.26; *d*, J_{PF} = 928 Hz]. Compound **36** was found to be completely absent when 5 equivalents of TBAF was employed in a solvent mixture which contains only tetrahydrofuran and pyridine [expt. #4 in Table 1]. On the other hand, when a large excess of water over TBAF was used, a 1: 1 mixture of **31** and **36** were found to have formed [expt. #1 in Table 1].

These findings (vide supra) are consistent with the proposed mechanism outlined in Scheme 3 which suggest that at least two equivalents of fluoride ion are required to give the phosphorodifluoridates **11b** or **11d** from

Table 1 : Fluoride treatments of O,O,S - thiophosphotriester (27) with TBAF trihydrateduring 30 min at 20 ° C.



27 31 32 36

Experiment #	TBAF $3H_2O$ (eq.)	THF-pyr-H_2O (v/v/v)	*Products* [a] 31	32	36
1	1.3	8:1:1	55	-	45
2	5.0	8:1:1	70	10	20
3	10.0	8:1:1	60	30	10
4	5.0	9:1	58	42	-

[a] Approximate ratios determined by ^{31}P-NMR

11. If the stoicheometry of TBAF is less or only slightly higher than this, water competes more successfully with the fluoride ions in the second step as the nucleophile [**11a** → **11b** or **11d**] to hydrolyse the initially formed thiophosphoromonofluoridate **11a** to the phosphorothioate **35**.

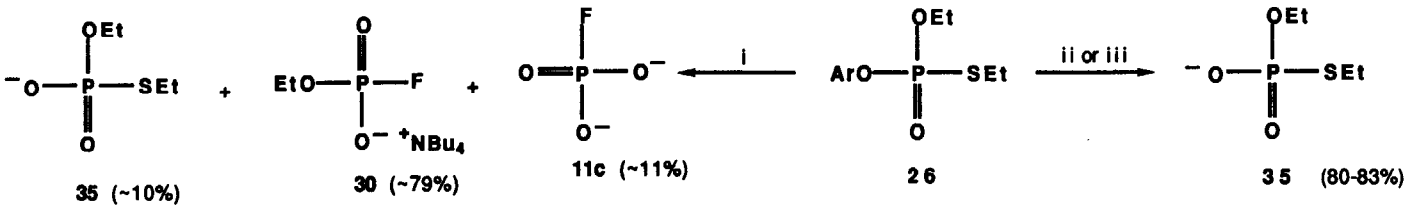
Above studies with the fluoride ion clearly showed the possibility of devising a new procedure for the synthesis of various O-alkylphosphoromonofluoridates. Several synthetic procedures have been earlier introduced[39-41] since the O-monoalkyl esters of monofluorophosphoric acid and its sodium salt have been found to be useful as cholinesterase inhibitor[40] and as an isosteric analogue of mononucleotides, with one less charge, to probe the biochemical transformations in biological experiments[42]. New synthesis of phosphorofluoridate analogues of nucleotides, particularly of ATP, phosphomonoesters, diesters or phosphocreatine, could be also useful in studying the metabolic processes by noninvasive NMR imaging techniques (Magnetic Resonance Tomography) taking advantage of 100% abundance of ^{19}F nucleus and its high inherent NMR sensitivity.

O,O,S-phosphorothioates **27-29** were thus synthesized in 82, 95, and 86% yield, respectively, using a standard condensation procedure (Scheme 7). They were then subjected to the treatment of fluoride ion, using the optimal condition devised in expt. #5 in Table 1 to give the n-tetrabutylammonium O-alkylphosphoromonofluoridates **31-34** (Scheme 7). It may be noted that the combined yields of phosphorofluoridates **31** and **32** from **27** is 85%. Compounds **33** and **34** were formed in 63 and 90% yield, respectively.

Monoalkylphosphoromonofluoridates are unstable in presence of a vicinal hydroxyl group in ribonucleosides. Earlier, it has been shown that simple monoalkylphosphoromonofluoridates are stable for several hours towards hydrolysis in dilute sodium hydroxide, ammonia and sulfuric acid[39]. It was therefore of interest to examine the stability of the 3′-phosphoromonofluoridate group exposed to the vicinal 2′-hydroxyl function. Thus, the 2′-O-pixyl group from compound **20** was removed by treatment with 80% aqueous acetic acid at room temperature (Scheme 6). When the reaction was quenched after 30 min by evaporation and neutralization, the observed ^{31}P signals corresponds to a mixture of the cyclic phosphate **21** [$\delta\ ^{31}P = +19.42$] and the isomeric 3′-phosphate **22** [$\delta\ ^{31}P = +1.12$] and 2′-phosphate **23** [$\delta\ ^{31}P = +0.34$] in an approximate ratio of 13 : 58 : 29, respectively. The cyclic phosphate **21** was isolated in 11% yield from this reaction. When the depixylation reaction was quenched after 12 h, only compounds **22** and **23** were found to have formed in 67 : 33 ratio [^{31}P-NMR], and were isolated as a chromatographically inseparable mixture in 66% yield. Our study thus clearly shows that the high stability of monoalkylphosphoromonofluoridates is completely annihilated by a vicinal free hydroxy function.

Stabilty of 5′-S-phosphorothioate linkage in a (3′ → 5′)di-ribonucleotide vicinal to a free 2′-hydroxy function. An important part of our study that remained to be answered was the question of the stability of the diribonucleoside 5′-S-phosphorothioate linkage vicinal to a free 2′-hydroxy function. Since this could not be settled using the fully protected dimer **11**, we synthesized the fully protected dimers **12** and **13** for this purpose (Scheme 1). As the first step, we removed the β-cyanoethyl group from dimers **12** and **13** by treatment with excess triethylamine in pyridine (Scheme 8). This provided us with the partial deprotected dimers **37** [$\delta\ ^{31}P = +14.6$] and **38** [$\delta\ ^{31}P = +16.3$]. Dimer **37** was not chromatographed through a silica gel column because of the lability of the 2′-O-pixyl group vicinal to the internucleotidyl phosphorothioate linkage during

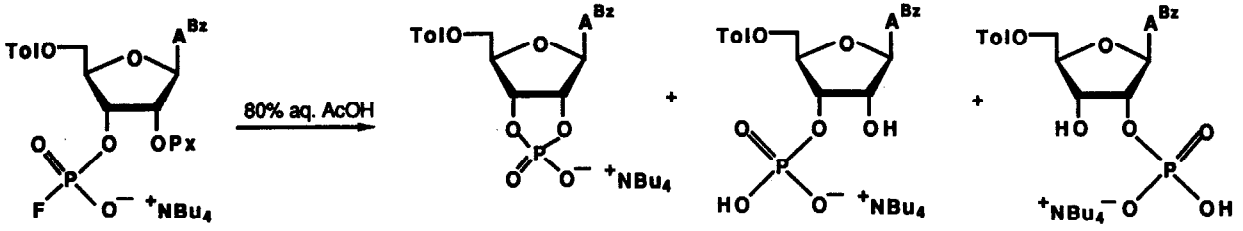
Scheme 5 : Degradation and hydrolysis of O-(2-chlorophenyl)-O,S-diethylthiophosphate (26)



35 (~10%) 30 (~79%) 11c (~11%) 26 35 (80-83%)

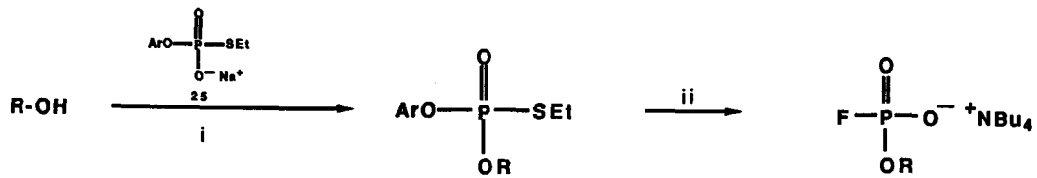
(i) TBAF . 3H$_2$O (2 eq.), THF-pyridine-water (8:1:1 v/v/v), 1 h at 20 °C ; (ii) Et$_3$N / H$_2$O / dioxane (2:1:1 v/v/v), 20 h at 20 °C ; (iii) TEAF (4 eq.) in THF-pyridine-water (5:1:4 v/v/v), 24 h at 50 °C

Scheme 6 : Cleavage of the 6-N-benzoyl-2′-O-pixyl-5′-O-toluoyladenosine 3′-phosphoromonofluoridate (20) upon removal of the 2′-O-pixyl group



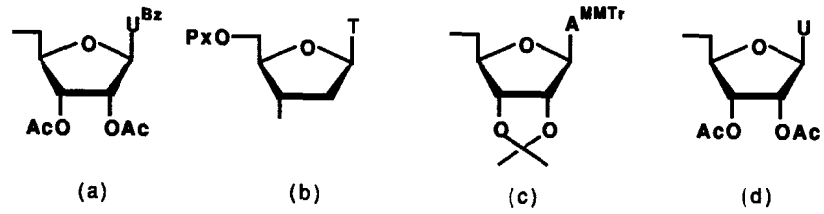
20 21 22 23

Scheme 7 : Preparation of O,O,S-thiophosphotriesters **26 - 29** and their conversion to the O-alkylphosphoromonofluoridates **30 - 34**



R = Et, (a), (b), or (c)

26 : R = Et	(88%)	
27 : R = (a)	(82%)	
28 : R = (b)	(95%)	
29 : R = (c)	(86%)	
30 : R = Et	(79%)	
31 : R = (a)	(49%)	
32 : R = (d)	(36%)	
33 : R = (b)	(63%)	
34 : R = (c)	(90%)	



(i) ROH (1 eq.), 26 (1.1 eq.), MS-NT (3 eq.) in pyridine, 1 h at 20 °C; (ii) TBAF . 3H_2O (5 eq.) in THF - pyridine (9:1 v/v), 30 min at 20 °C

such manipulations. Dimer **38** could be however easily purified and collected in 85% yield. Next, the 2′-O-protecting groups were removed from dimers **37** and **38** (Scheme 9). The ^{31}P-NMR examination of the crude reaction mixtures, resulted from deprotection of the dimer **37** with 80% aqueous acetic acid for 30 min at room temperature, revealed the presence of the dimer **39** [δ ^{31}P = +17.26] along with 2′,3′-cyclic phosphate **40** [δ ^{31}P = +18.33] in 76 : 24 ratio. Similarly, the dimer **38**, upon treatment with TBAF for 12 h at room temperature, gave also the ^{31}P signals corresponding to the dimer **39** [δ ^{31}P = +17.33] and the 2′,3′-cyclic phosphate **21** [δ ^{31}P = +18.50] (see also Scheme 6) in 78 : 22 ratio. Our repeated attempts to purify the dimer **39** by preparative thin layer chromatography steadily degraded the dimer into **40** (from **37**) and **21** (from **38**) and other products **18** and **19** as verified by ^{31}P and ^{1}H-NMR spectrum (Scheme 9, see experimental). This indicates that the dimer **39** is only transiently stable both under acidic and neutral conditions.

We have subsequently tried to carry out a "controlled" deprotection of **37** and **38**. The dimer **37** was therefore stirred with silica gel in a mixture of acetonitrile and water at pH ~ 5 (Scheme 9) for 4 days at 40 °C, and the products that were isolated by preparative thin layer chromatography were cyclic phosphate **40** (75%) and the disulfides **18** (44%) and **19** (16%). Although the dimer **39** (~10%) was observed on TLC but all attempts to purify this led to its further decomposition. Similarly, the dimer **38** upon TBAF treatment showed also the formation of the dimer **39** which decomposed much more sluggishly in the crude reaction mixture [^{31}P-NMR] due to the presence of TBAF. When the crude mixture was made free of TBAF and subjected to the silica gel treatment (see experimental), it decomposed to give cyclic phosphate **21** (58%), and the disulfides **18** (11%) and **19** (29%). Although some undecomposed **39** (~5-10%) was again observed in this particular case on TLC but all our attempts to isolate it in a pure form were unsuccessful.

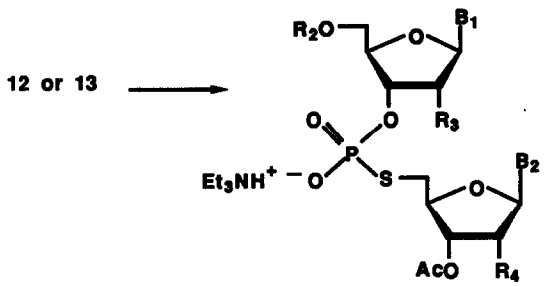
These experiments thus clearly show that the phosphorothioate linkage in *ribo*nucleoside-3′-O-PO_2-S-5′-*ribo*nucleoside are indeed very labile both under acidic and neutral conditions due to the participation of the vicinal 2′-hydroxy function.

This was further confirmed by the deprotection of **14** to give thymidyl(3′→ 5′)5′-thiothymidine [d(TpsT)] **41**[13-16] (51%) [δ ^{31}P = +19.1 as the sole signal], by treatment with concentrated ammonia (12 h at 20 °C), and 80% aqueous acetic acid (6 h at 20 °C) followed by a purification through a DEAE Sephadex chromatography. The structure of **41** was finally corroborated by detailed spectroscopic studies (see experimental). Thus, the isolation of pure, well characterized thymidyl(3′→ 5′)5′-thiothymidine **41** clearly show that the phosphorothioate linkage [-3′-O-PO_2-S-5′-] in *2′-deoxyribonucleotides* is indeed stable, and *the lability of the oligonucleotide-5′-S*-phosphorothioate *linkage in ribonucleoside-3′-O-PO_2-S-5′-ribonucleoside* **39** *is due to the presence of the vicinal 2′- hydroxyl function.* Furthermore, the unambiguous strategy for the synthesis of **41** by the phosphotriester methodology proved to be a more efficient procedure for the chemoselective synthesis of fully deprotected oligodeoxyribonucleoside-5′-S-phosphorothioates such as **41** than the ones previously reported[13-16].

EXPERIMENTAL

^{1}H-NMR spectra were recorded in δ scale with Jeol FX 90 Q and JNM GX 270 spectrometers at 90 and 270 MHz respectively, using TMS or acetonitrile (set at 2.0 ppm) as internal standards. ^{31}P-NMR spectra were recorded at 36 MHz in the same solvent using 85 % phosphoric acid as external standard. UV-absorption

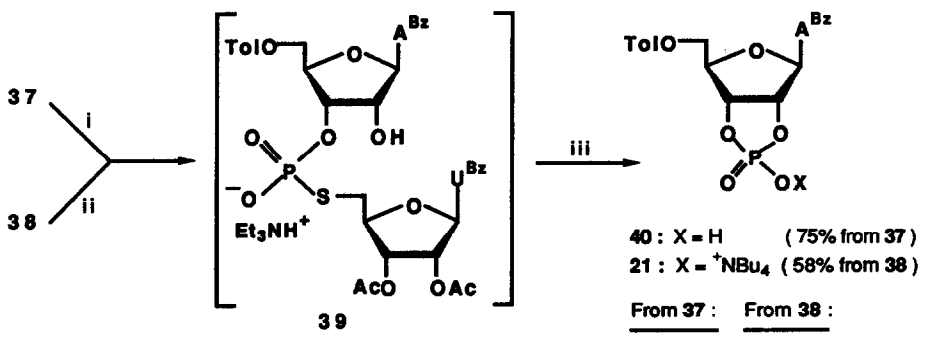
Scheme 8 : Partial deprotection of fully protected dimers 12 & 13 by action of triethylamine (15 eq.) *in pyridine, 4 h at 20 °C*



37 : $B_1 = A^{Bz}$, $B_2 = U^{Bz}$, R_2 = Tol, R_3 = O-Px, R_4 = OAc

38 : $B_1 = A^{Bz}$, $B_2 = U^{Bz}$, R_2 = Tol, R_3 = O-TBDMS, R_4 = OAc

Scheme 9 : Products formed upon the removal of the 2′-protecting group from 37 & 38



40 : X = H (75% from 37)

21 : X = $^{+}NBu_4$ (58% from 38)

From 37 :	From 38 :
39 (11%)	39 (16%)
18 (44%)	18 (11%)
19 (16%)	19 (29%)

(i) 80% aq. AcOH, 30 min at 20 °C; (ii) TBAF. $3H_2O$ (3 eq.), THF, 12 h at 20 °C; (iii) MeCN - H_2O (5:1 v/v), silica gel, pH ~5, 3 - 4 days at 40 °C;

spectra were recorded with a Varian Cary 2200 spectrophotometer. A Jeol DX 303 instrument was used for recording high resolution mass spectra. TLC was carried out using pre-coated silica gel F_{254} plates in the following solvent systems: dichloromethane-methanol mixtures: (A) 98:2 (v/v), (B) 95:5 (v/v), (C) 90:10 (v/v), (D) 80:20 (v/v), and (E) 70:30 (v/v); acetonitrile-water mixtures: (F) 7:1 (v/v), (G) 9:1 (v/v). The salts of the thiophosphoro-O-monoesters and thiophosphoro-O,S-diesters were visualized as yellow-brown spots by spraying with an acidic solution of $PdCl_2$[30]. The column chromatographic separations were carried out using Merck G 60 silica gel and preparative thin layer separations were carried out using pre-coated 200 x 200 x 2 mm silica gel F_{254} plates.

O-(2-chlorophenyl)-bis-triethylammonium phosphorothioate (1). This was prepeared using a modified literature procedure[30]. Barium acetate (9.75 g, 38.2 mmol) was dissolved in distilled water (120 ml). O-(2-chlorophenyl) phosphorodichloridothioate[29] (5.0 g, 19.1 mmol) was then added under vigorous stirring at 0 °C, followed by dropwise addition of triethylamine (10.6 ml, 76.2 mmol). The reaction mixture was warmed up to 20 °C, and dioxane was added until the solution became clear. After stirring for 1 h at 20 °C, the resulting white precipitate was collected by filtration and then washed with water, ethanol and diethylether, to give barium O-(2-chlorophenyl)phosphorothioate in a quantitative yield (6.84 g) as a white powder. The bariumsalt (1 g, 2.78 mmol) was resuspended in distilled water (25 ml). To this suspension, an aqueous solution of triethylammonium sulfate (0.53 M, 5 ml, pH 7,) was added. After stirring for 5 min,the resulting barium sulfate was removed by filtration and the filtrate evaporated *in vacuo* , followed by co-evaporations with acetonitrile (2 x 20 ml) and toluene (2 x 20 ml), to give an oil (740 mg, 63%). R_f: 0.47 (F); ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 7.79-6.97 (m, 4H) arom.; 3.32-3.04 (m, 12H) -CH_2 in triethylammonium.; 1.37-1.23 (m, 18H) -CH_3 in triethylammonium.; ^{31}P-NMR ($CDCl_3$ + CD_3OD) : +50.45 ppm. The analogous disodium salt was obtained as a white powder in 94% yield, by treating the bariumsalt with sodium sulfate instead of triethylammonium sulfate. R_f: 0.36 (F); ^{1}H-NMR (D_2O) : 7.62-6.93 (m, 4H) arom.; ^{31}P-NMR (D_2O) : +41.41 ppm.

O-(2-cyanoethyl)-bis-triethylammonium phosphorothioate (2). This compound was also prepeared using a modified literature procedure[31]. Thiophosphorylchloride (10.2 ml, 0.1 mol) was dissolved in anhydrous diethylether (100 ml) in a three-necked flask equipped with a thermometer and a pressure-equalizing dropping funnel. The solution was cooled to -10 °C and then a mixture of anhydrous pyridine (6.8 ml, 0,1 mol) and 3-hydroxypropionitrile (8.1 ml, 0.1 mol) was added dropwise with vigorous stirring in such a rate that the temperature did not exceed -10 °C. After the addition was complete, the reaction mixture was stirred for 3h at -10 °C. The white suspension was then poured slowly with stirring into a mixture of water (375 ml), pyridine (40 ml) and ice (150 g). After stirring for 15 min, a concentrated aqueous solution of barium acetate (51 g, 0.2 mol) was added and the resulting opalescent mixture was stirred for 1 h at 20 °C. The white precipitate was removed by filtration and the filtrate was slowly diluted with 2 L of 95% ethanol with stirring, and the resulting milky suspension was stirred for 2 h at 0 °C. The suspension was filtered and the white solid was washed with 50% ethanol and then with 95% ethanol. Vacuum drying over P_2O_5 gave a white powder (12.1 g). This solid material was further purified from traces of inorganic phosphorothioate in the same way as prescribed in the literature[16] to give of barium O-(2-cyanoethyl)phosphorothioate as a white solid (10.9 g, 36%). R_f: 0.22 (F); ^{31}P-NMR (H_2O) : + 42.51 ppm. The bariumsalt (1 g, 3.31 mmol) was dissolved in distilled water (25 ml) and this solution was treated with triethylammoniun sulfate (993 mg, 3.31 mmol) in the same way as for preparation of compound **1**. Yield 887 mg (73%) as an oil. R_f: 0.26 (F); ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 4.21(dt, J = 6.6 Hz, $^3J_{HCOP}$ = 7.6 Hz, 2H) -POCH_2CH$_2$CN ; 2.78 (t, 2H) -POCH$_2$CH_2CN ; ^{31}P-NMR ($CDCl_3$ + CD_3OD) : +56.25 ppm.

3-N-benzoyl-2′,3′-di-O-acetyl-5′-O-(methanesulfonyl)uridine (6). 3-N-benzoyl-2′,3′-di-O-acetyluridine (3.3 g, 7.6 mmol) was dried by co-evaporations with pyridine. The dry residue was taken up in the same solvent (50 ml), methylsulfonyl chloride (1.31 g, 11.4 mmol) was then added and the mixture was stirred for 2 h at 20 °C. The reaction was quenched by pouring into saturated sodium hydrogen carbonate solution and extracting with dichloromethane (3 x 100 ml). The organic phase was dried and purified by short silica gel column chromatography, using the following mixture of solvents as eluents: light petroleum - dichloromethane (25:75 v/v), dichloromethane, and then ethanol - dichloromethane (1:99 v/v). Appropriate fractions were pooled and evaporated to give a white foam (3.34 g, 86%). R_f: 0.36 (B); ^{1}H-NMR ($CDCl_3$) : 8.00-7.41 (m, 6H) arom. & H-6 ; 5.99 (d, $J_{1',2'}$ = 5.1 Hz, 1H) H-1′; 5.91 (d, J = 8.3 Hz, 1H) H-5 ; 5.40-5.34 (m, 2H) H-2′, H-3′; 4.54-4.34 (m, 3H) H-4′, H-5′, H-5′′; 3.07 (s, 3H) -SO_2CH_3 ; 2.11, 2.07 (2 x s, 2 x 3H) 2 x -$COCH_3$.

3-N-benzoyl-3′-O-acetyl-5′-O-(methanesulfonyl)thymidine (7). 3-N-benzoyl-3′-O-acetylthymidine (778 mg, 2 mmol) was treated with methanesulfonyl chloride (344 mg, 3 mmol) in the same way as for the preparation of compound **6**. Yield 784 mg (79%). R_f: 0.47 (B).^{1}H-NMR ($CDCl_3$) : 7.99-7.39 (m, 6H) arom. & H-6 ; 6.36 (dd, $J_{1',2'}$ = 8.8 Hz, $J_{1',2''}$ = 5.9 Hz, 1H) H-1′; 5.29 (m, 1H) H-3′; 4.63 (dd, $J_{5',5''}$ = 11.2 Hz,

$J_{5',4'}$ = 3.2 Hz, 1H) H-5'; 4.46 (dd, 1H) H-5''; 4.21 (m, 1H) H-4'; 3.10 (s, 3H) -SO_2CH_3 ; 2.42 (m, 2H) H-2'; 2.10 (s, 3H) -$COCH_3$; 1.98 (d, J = 1.2 Hz, 3H) 5-CH_3.

O-(2-chlorophenyl)-S-5'-(3-N-benzoyl-2',3'-di-O-acetyl-5'-deoxyuridyl)phosphorothioate triethylammonium salt (8). Compound **6** (766 mg, 1.5 mmol) and compound **1** (345 mg, 0.75 mmol) were mixed together and dried by co-evaporation with toluene (2 x 10 ml). The residue was then dissolved in dry dimethylformamide (15 ml) and dry triethylamine (1.6 ml) was added.The mixture was then stirred for 4 days at 20 °C. The reaction mixture was evaporated at 40 °C *in vacuo*, followed by co-evaporation with toluene. The residue was loaded onto a short silica gel column and after a stepwise increase of the ethanol percentage in dichloromethane, the title compound was eluated with ~ 9% ethanol - dichloromethane mixture. Yield 250 mg (46%) as a white solid after precipitation from hexane.R_f: 0.42 (D); ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 8.03-7.01 (m, 10H) arom. & H-6 ; 6.03 (d, $J_{1',2'}$ = 6.1 Hz, 1H) H-1'; 5.85 (d, J = 8.1 Hz, 1H) H-5 ; 5.56-5.27 (m, 2H) H-2', H-3'; 4.39 (m, 1H) H-4'; 3.38-2.99 (m, 8H) H-5', H-5''& CH_2 of triethylammonium ; 2.08, 1.98 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.34 (t, J = 7.3Hz, 9H) CH_3 of triethylammonim ; ^{31}P-NMR ($CDCl_3$ + CD_3OD): +12.46 ppm.

O-(2-cyanoethyl)-S-5'-(3-N-benzoyl-2',3'-di-O-acetyl-5'-deoxyuridyl)phosphorothioate triethyl ammonium salt (9). Compounds **6** (766 mg, 1.5 mmol) and **2** (275 mg, 0.75 mmol) were treated in an identical manner as described for the preparation of **8**, except that the reaction mixture was stirred for 3 days at 20 °C. The title compound was obtained in 68% yield (350 mg) as a white foam after purification by short silica gel column chromatography and precipitation from hexane (the title compound was eluated with ~ 9% ethanol - dichloromethane mixture). R_f: 0.30 (D); ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 8.04-7.41 (m, 6H) arom. & H-6 ; 6.09 (d, $J_{1',2'}$ = 6.1 Hz, 1H) H-1'; 5.93 (d, J = 8.3 Hz, 1H) H-5 ; 5.63-5.37 (m, 2H) H-2', H-3'; 4.41 (m, 1H) H-4'; 4.16 (dt, J = 6.6 Hz, $^3J_{HCOP}$ = 8.3 Hz, 2H) -POCH_2CH_2CN ; 3.23-2.71 (m, 10H) H-5', H-5'', CH_2 of triethylammonium & -POCH$_2CH_2$CN ; 2.09, 1.99 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.35 (t, J = 7.6 Hz, 9H) CH_3 of triethylammonium ; ^{31}P-NMR ($CDCl_3$ + CD_3OD) : + 15.94 ppm.

O-(2-cyanoethyl)-S-5'-(3-N-benzoyl-3'-O-acetyl-5'-deoxythymidyl)phosphorothioate triethyl ammonium salt (10). Compounds **7** (443 mg, 0.95 mmol) and **2** (174 mg, 0.47 mmol) were treated in an identical manner as for the preparation of compound **8**. They were dissolved in dry dimethylformamide (10 ml), and dry triethylamine (1 ml) was added. The reaction mixture was stirred for 4 days at 20 °C. The title compound was eluated with ethanol - dichloromethane mixture (10:90 v/v). Yield: 107 mg (58%) as a white foam after precipitation from hexane. R_f: 0.31 (D) ; ^{1}H-NMR ($CDCl_3$ + CD_3OD): 7.96-7.40 (m, 6H) arom.& H-6 ; 6.25 (t, 1H) H-1'; 5.46 (m, 1H) H-3'; 4.37-4.07 (m, $^3J_{HCOP}$ = 9.5 Hz, 3H) H-4' & -POCH_2CH_2CN ; 3.27-2.70 (m, 10H) H-5', H-5'', CH_2 of triethylammonium & -POCH$_2CH_2$CN; 2.39 (m, 2H) H-2'; 2.07 (s, 3H) -$COCH_3$; 1.99 (s, 3H) 5-CH_3 ; 1.35 (t, J = 7.1 Hz, 9H) CH_3 of triethylammonium. ^{31}P-NMR ($CDCl_3$ + CD_3OD) : + 15.69 ppm.

Sodium O-(2-chlorophenyl)-S-ethylphosphorothioate (25). Disodium O-(2-chlorophenyl) phosphorothioate (2 g, 7.46 mmol) was suspended in dry dimethylformamide (10 ml), then ethylbromide (8.13 g, 74.6 mmol) was added and the mixture was stirred for 12 h at 20 °C. The volatile matters were evaporated *in vacuo* and the residue co-evaporated with toluene.The residue was triturated with a small amount of a dichloromethane - ethanol mixture (99:1 v/v) and the insoluble materials were removed by filtration. The filtrate was then poured dropwise into a cold mixture of petroleum ether and diethylether (1:1 v/v) (400 ml) and the white precipitate was collected by filtration and washed with the same solvent mixture.Yield 1.54 g (75%). R_f: 0.32 (E); ^{1}H-NMR (CD_3OD): 7.71-6.93 (m, 4H) arom. ; 2.86 (dq, J = 7.3 Hz, $^3J_{HCSP}$ = 12.2 Hz, 2H) -PSCH_2CH_3; 1.26 (t, 3H) -PSCH$_2CH_3$; ^{31}P-NMR (CD_3OD): +15.67 ppm.

Preparation of O,O,S-phosphorothioates : 26, 27, 28 and 29. *A general procedure:* Dry alcohol (1 mmol) and dry **25** (1.1 mmol) were dissolved in dry pyridine (10 ml). 1-Mesitylenesulfonyl-3-nitro-1,2,4-triazole (3 mmol) was then added and the reaction mixture was stirred for 1 h at 20 °C. It was worked up in the usual way to give a pyridine-free gum. Each triester was then isolated as an inseparable mixture of the two diastereomers by short silica gel column chromatography using dichloromethane and dichloromethane - ethanol (99:1 v/v) as sequence of mobile phases. **Compound 26.** Yield 195 mg (82%). R_f: 0.47 (A) ; ^{1}H-NMR ($CDCl_3$) : 7.62-7.10 (m, 4H) arom. ; 4.39 (dq, J = 6.8 Hz, $^3J_{HCOP}$ = 9.0 Hz, 2H) -POCH_2CH_3 ; 2.96 (dq, J = 7.3 Hz, $^3J_{HCSP}$ = 15.6 Hz, 2H) -PSCH_2CH_3 ; 1.43, 1.34 (2 x t, 2 x 3H) -POCH$_2CH_3$ & -PSCH$_2CH_3$. ^{31}P-NMR ($CDCl_3$) : +25.22 ppm. **Compound 27.** Yield 547 mg (77%). R_f: 0.47 (B) ; ^{1}H-NMR ($CDCl_3$) : 8.01-7.16 (m, 10H) arom. & H-6 ; 6.18 (d, $J_{1',2'}$ = 6.4 Hz, 1H) H-1'; 5.89, 5.82 (2 x d, J = 8.3 Hz, 1H) H-5 ; 5.52- 5.21 (m, 2H) H-2', H-3'; 4.60-4.35 (m, 3H) H-4', H-5' & 5''; 3.01 (dq, J = 7.8 Hz, $^3J_{HCSP}$ = 16.1 Hz, 2H) PSCH_2CH_3; 2.13, 2.04 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.37 (t, 3H) -PSCH$_2CH_3$. . ^{31}P-NMR ($CDCl_3$) : +26.61 & +27.15 ppm. **Compound 28.** Yield 730 mg (95%). R_f: 0.27 (B) ; ^{1}H-NMR ($CDCl_3$) : 8.02 (br, 1H) NH ; 7.65 (d, $J_{6,5\text{-}CH3}$ = 1.0 Hz, 1H) H-6 ; 7.47-7.01 (m, 17H) arom.; 6.45 (t, 1H) H-1'; 5.33 (m, 1H) H-3'; 4.30 (m, 1H) H-4'; 3.27 (m, 2H) H-5' & 5''; 3.14-2.22 (m, 4H) H-2' & -PSCH_2CH_3 ; 1.64 (s, 3H) 5-

CH_3 ; 1.29 (t, J = 7.3 Hz, 3H) -PSCH$_2$*CH$_3$* ; ^{31}P-NMR ($CDCl_3$): +25.47 & +25.65 ppm. **Compound 29.** Yield 696 mg (82%). R_f: 0.28 (A) ; ^{1}H-NMR ($CDCl_3$) : 8.04 (s, 1H) H-8 ; 7.89 (s, 1H) H-2 ; 7.53-6.73 (m, 18H) arom.; 6.11 (d, $J_{1',2'}$ = 2.4 Hz, 1H) H-1'; 5.47-5.29 (m, 1H) H-2'; 5.14 (m, 1H) H-3'; 4.57-4.19 (m, 3H) H-4',H-5' & 5''; 3.77 (s, 3H) -OCH_3 ; 2.87 (dq, J = 7.8 Hz, $^3J_{HCSP}$ = 16.1 Hz, 2H) -PS*CH$_2$*CH$_3$; 1.61, 1.37 (2 x s, 2 x 3H) isopropylidene-CH_3 ; 1.23 (dt, 3H) -PSCH$_2$*CH$_3$*. ^{31}P-NMR ($CDCl_3$) : +26.14 ppm.

Hydrolysis of O-(2-chlorophenyl)-O,S-diethylphosphorothioate (26). *(a) with triethylamine / water / dioxane.* Compound **26** (45 mg, 0.161 mmol) was dissolved in the mentioned solvent mixture (2:1:1 v/v/v, 1 ml) and stirred for 20 h at 20 °C. The volatile matters were removed by evaporation in vacuo, the residue co-evaporated with toluene and the sole product was isolated by preparative thin layer chromatography using solvent F as the mobile phase.The isolated single product was identified as O,S-diethylphosphorothioate (**35**) .Yield 22 mg (80%). R_f: 0.17 (E); ^{1}H-NMR (D_2O) : 3.90 (dq, J = 7.8 Hz, $^3J_{HCOP}$ = 8.1 Hz, 2H) -PO*CH$_2$*CH$_3$; 2.68 (dq, J = 8.3 Hz, $^3J_{HCSP}$ = 12.7 Hz, 2H) -PS*CH$_2$*CH$_3$; 1.21 (2 x t, 6H) -POCH$_2$*CH$_3$* & -PSCH$_2$*CH$_3$* ; ^{31}P-NMR (D_2O): +20.74 ppm. *(b) with triethylammonium fluoride.* Compound **26** (47 mg, 0.168 mmol) was dissolved in a tetrahydrofuran - pyridine - water mixture (5:1:4 v/v/v, 1 ml). Triethylammonium fluoride (72 mg, 0.596 mmol) was then added and the reaction mixture was stirred for 24 h at 50 °C. The product **35** was isolated in the same way as described under reaction *(a)*. Yield 23.6 mg (83%). R_f: 0.17 (E). ^{1}H-NMR (D_2O) and ^{31}P-NMR (D_2O) were identical to the product obtained in reaction *(a)*

Fluoride treatment of O-(2-chlorophenyl)-O-5'-(3-N-benzoyl-2',3'-di-O-acetyl-uridyl)-S-ethyl phosphorothioate (27). Compound **27** (100 mg, 150 μmol) was dissolved in THF-pyridine-water mixture (8:1:1, v/v/v, 1.5 ml) and then TBAF trihydrate was added in one portion and the mixture was stirred for 30 min at 20 °C. The volatile matters were removed by evaporation *in vacuo* and the residue co-evaporated with toluene. The products formed were separated by TLC using the solvent system G. **3-N-benzoyl-2',3'-di-O-acetyluridine-5'-phosphoromonofluoridate n-tetrabutyl ammonium salt (31).** Yield 55 mg (49%). R_f: 0.55 (G) ; UV (95% EtOH) : λ_{max} 253 nm ; ^{1}H-NMR ($CDCl_3$) : 8.27-7.39 (m, 6H) arom. & H-6 ; 6.22 (d, $J_{1',2'}$ = 6.3 Hz, 1H) H-1'; 5.94 (d, J = 8.3 Hz, 1H) H-5 ; 5.59-5.31 (m, 2H) H-2', H-3'; 4.43-4.17 (m, 3H) H-4', H-5' & 5'' ; 3.42-3.08 (m, 8H) $^+$N(*CH$_2$*CH$_2$CH$_2$CH$_3$)$_4$; 2.05, 1.95 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.60-0.87 (m, 28H) $^+$N(CH$_2$*CH$_2$CH$_2$CH$_3$*)$_4$; ^{31}P-NMR ($CDCl_3$) : +6.32 & -19.26 ppm ; d, J_{PF} = 928 Hz ; MS (FAB^-) : calc.for (M-Bu$_4$N$^+$)$^-$ 513.0710, found 513.0713. **2',3'-di-O-acetyluridine-5'-phosphoromonofluoridate n-tetrabutylammonium salt (32).** Yield 35 mg (36%). R_f: 0.36 (G) ; UV (95% EtOH) : λ_{max} 258 nm ; ^{1}H-NMR ($CDCl_3$) : 8.04 (d, J = 8.3 Hz, 1H) H-6 ; 6.26 (d, $J_{1',2'}$ = 6.6 Hz, 1H) H-1'; 5.82 (d, 1H) H-5 ; 5.56-5.32 (m, 2H) H-2', H-3'; 4.34-4.16 (m, 3H) H-4', H-5' & 5''; 3.42-3.08 (m, 8H) $^+$N(*CH$_2$*CH$_2$CH$_2$CH$_3$)$_4$; 2.12, 2.03 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.60-0.84 (m, 28H) $^+$N(CH$_2$*CH$_2$CH$_2$CH$_3$*)$_4$; ^{31}P-NMR ($CDCl_3$) : +6.49 & -19.11 ppm ; d, J_{PF} = 929 Hz ; MS (FAB^-) : calc.for (M-Bu$_4$N$^+$)$^-$ 409.0449, found 409.0432. **O-5'-(3-N-benzoyl-2',3'-di-O-acetyl-uridyl)-S-ethylphosphorothioate n-tetrabutylammonium salt (36).** Yield 26 mg (43%) ; R_f: 0.48 (G) ; UV (95% EtOH) : λ_{max} 252 nm; ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 8.26 (d, $J_{5,6}$ = 8.3 Hz, 1H) H-6 ; 8.00-7.48 (m, 5H) arom.; 6.24 (d, $J_{1',2'}$ = 5.4 Hz, 1H) H-1'; 6.02 (d,1H) H-5; 5.53 (m, 2H) H-2',H-3'; 4.34-4.19 (m, 3H) H-4', H-5', H-5'' ; 3.33-3.15 (m, 8H) $^+$N(*CH$_2$*CH$_2$CH$_2$CH$_3$)$_4$; 2.81 (dq, $^3J_{HCSP}$ = 13.2 Hz, 2H) -PS*CH$_2$*CH$_3$; 2.10, 1.99 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.77-1.26 (m, 19H) $^+$N(CH$_2$*CH$_2$CH$_2$*CH$_3$)$_4$ & -PSCH$_2$*CH$_3$* ; 1.00 (t, 12H) $^+$N(CH$_2$CH$_2$CH$_2$*CH$_3$*)$_4$; ^{31}P-NMR ($CDCl_3$ + CD_3OD) : +17.04 ppm.

A general procedure for the preparation of n-tetrabutylammonium phosphorofluoridate O-monoesters. The O,O,S-phosphorothioate **28** or **29** (150 μmol) was dissolved in a mixture of tetrahydrofuran - pyridine (9:1 v/v, 3 ml). To this solution, TBAF trihydrate (0.71 mmol) was added and stirred for 30 min at 20 °C. A distinct odor of ethanethiol evolved during the reaction. The volatile matters were removed by evaporation *in vacuo* and the residue co-evaporated with toluene. Compounds **33** or **34** was isolated by short silica gel column chromatography using a step-gradient of dichloromethane-ethanol (93:7 - 86:14 v/v) mixture. **5'-O-pixylthymidine 3'-phosphoromonofluoridate n-tetrabutylammonium salt (33).** Yield 76 mg (63%). R_f: 0.45 (G) ; UV (95% EtOH) : λ_{max} 244, 263 (sh.), 281 (sh.) and 291 nm ; ^{1}H-NMR ($CDCl_3$): 7.69 (s, 1H) H-6 ; 7.41-7.06 (m, 13H) arom.; 6.40 (dd, $J_{1',2'}$ = 5.0 Hz, 1H)H-1'; 5.07 (m, 1H) H-3'; 4.22 (m, 1H) H-4'; 3,42-3.11 (m, 10H) H-5' & 5'', $^+$N(*CH$_2$*CH$_2$CH$_2$CH$_3$)$_4$; 2.76-2.28 (m, 2H) H-2' ; 2.19 (s, 3H) 5-CH_3 ; 1.83-0.87 (m, 28H) $^+$N(CH$_2$*CH$_2$CH$_2$*CH$_3$)$_4$; ^{31}P-NMR ($CDCl_3$) : +5.49 & -20.19 ppm ; d, J_{PF} = 931.5 Hz ; MS (FAB^-) calc.for (M-Bu$_4$N$^+$)$^-$ 579.1332, found 579.1332. **6-N-(4-methoxytrityl)-2',3'-O-isopropylideneadenosine-5'-phosphoromonofluoridate n-tetrabutyl**

ammonium salt (34). Yield 122 mg (90%). R_f: 0.70 (D) ; UV (95% EtOH) : λ_{max} 268 (sh.), 274, 283 (sh.) nm ; ^{1}H-NMR ($CDCl_3$ + CD_3OD): 8.33 (s, 1H) H-8 ; 8.04 (s, 1H) H-2 ; 7.36-6.72 (m, 14H) arom.; 6.20 (d, $J_{1',2'}$ = 3.4 Hz, 1H) H-1′ ; 5.23 (dd, $J_{2',3'}$ = 6.1 Hz, 1H) H-2′; 5.06 (dd, $J_{3',4'}$ = 1,5 Hz, 1H) H-3′ ; 4.49 (m, 1H) H-4′; 4.25-4.09 (m, $J_{5',5''}$ = 5.6 Hz, 2H) H-5′ & 5′′; 3.77 (s, 3H) -OCH_3; 3.38-3.15 (m, 8H) $^+N(CH_2CH_2CH_2CH_3)_4$; 1.84-1.25 (m, 22H) $^+N(CH_2CH_2CH_2CH_3)_4$ & isopropylidene -CH_3 ; 1.00 (t, 12H) $^+N(CH_2CH_2CH_2CH_3)_4$; ^{31}P-NMR ($CDCl_3$ + CD_3OD): +6.72 & -18.75 ppm ; d, J_{PF} = 924 Hz. MS (FAB$^-$): calc.for $(M\text{-}Bu_4N^+)^-$ 660.2023, found 660.2054.

Fully protected O,O,S-phosphorothioate (24). This compound was prepared in the same way as described for the preparation of compound **11**, except the bistriazolide treatment was omitted. Amounts employed were 106 mg (0.161 mmol) of compound **8**, 11.6 µl (0.198 mmol) of dry ethanol and 244 mg (0.83 mmol) of MSNT. After usual work up and purification by short silica gel column chromatography (dichloromethane-ethanol, 99:1 v/v as mobile phase), the title compound was collected in 89% yield (96 mg), as an inseparable mixture of the two diastereomers. R_f: 0.46 (B) ; ^{1}H-NMR ($CDCl_3$): 7.98-7.13 (m, 11H) arom. & H-6 ; 5.94-5.80 (m, 2H) H-1′, H-5 ; 5.45-5.22 (m, 2H) H-2′, H-3′; 4.54-4.19 (m, $^3J_{HCOP}$ = 9.5 Hz, 3H) H-4′ & -POCH_2CH_3 ; 3.44 (d, $J_{5',5''}$ = 5.9 Hz, 1H) H-5′ ; 3.26 (d, 1H) H-5′′ ; 2.09, 2.04, 2.03 (3 x s, 2 x 3H) 2 x -$COCH_3$; 1.43 (t, J = 7.1 Hz, 3H) -$POCH_2CH_3$; ^{31}P-NMR ($CDCl_3$) : +23.45 ppm.

Fluoride treatment of the fully protected O,O,S-phosphorothioate (24). This experiment was carried out in the same manner as for **11**, applying 96 mg (144 µmol) of **24** and 93 mg (294 µmol) of TBAF trihydrate. After the removal of the volatile matters, the pyridine-free residue was taken up in dichloromethane (5 ml) and extracted with water (3 x 5 ml).Both phases were collected separately and evaporated to dryness. The residue from the organic phase was subjected to preparative thin layer chromatography, using solvent B as mobile phase. Following products were characterized: **O-ethylphosphoromonofluoridate n-tetrabutylammonium salt (30)**. Found in the residue after evaporation of the aqueous phase,which also contains some of the excess TBAF. ^{1}H-NMR ($CDCl_3$): 4.00 (dq, J = 7.6 Hz, $^3J_{HCOP}$ = 7.6 Hz, $^4J_{HCOPF}$ = 2.4 Hz, 2H) -POCH_2CH_3 ; 1.25 (t, 3H) -$POCH_2CH_3$; 3.40-3.22, 1.84-1.33, 1.01 (m) $^+N(CH_2CH_2CH_2CH_3)_4$; ^{31}P-NMR ($CDCl_3$) : +6.41 & -18.99 ppm, d, J_{PF} = 921.6 Hz. **Compound 16** : Yield 18 mg (23%) ; **Compound 17** : Yield 19 mg (29%) ; **Compound 18** : Yield 1.3 mg (2%) ; **Compound 19** : Yield 6 mg (10%).

Fully protected dimer ApsU (11). A mixture of compound **8** (281 mg, 0.38 mmol) and 6-N-benzoyl-2′-O-pixyl-5′-O-toluoyladenosine[43] **3** (264 mg, 0.35 mmol) was co-evaporated with dry pyridine and then redissolved in dry pyridine (4 ml). 1-Mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) (518 mg, 1.75 mmol) was then added and the reaction mixture stirred for 4 h at 20 °C. Then 2-chlorophenylphosphorobis(1,2,4-triazolide) (0.2 M in acetonitrile, 3.5 ml , 2 equiv.) was added and stirred for 30 min,after which the mixture was poured into saturated hydrogen carbonate solution and extracted with dichloromethane. The pyridine free gum obtained after evaporation in vacuo of the organic phase and co-evaporation with toluene was purified by short silica gel column chromatography,using dichloromethane and dichloromethane - ethanol (99:1 v/v) as mobile phases (containing 1 drop of pyridine / 10 ml solvent). The yield of an inseparable mixture of two diastereomers was 350 mg (73%) after precipitation from cold hexane. R_f : 0.50 (B) ; ^{1}H-NMR ($CDCl_3$): [*mixture of R_P and S_P*] 8.39, 8.33 (2 x s, 1H) AH-8; 8.03-6.29 (m, 35H) arom. & AH-2, UH-6; 6.16-5.80 (m, 3H) AH-1′, UH-1′, UH-5; 5.50-5.29 (m, 3H) AH-2′, UH-1′, UH-3′; 4.87-4.27 (m, 4H) AH-3′, AH-4′, AH-5′ & 5′′, UH-4′ ; 3.55 (m, 1H) UH-5′; 3.35 (m, 1H) UH-5′′; 2.49, 2.47 (2 x s, 3H) -COPhCH_3 ; 2.11, 2.07, 2.04 (3 x s, 6H) 2 x -$COCH_3$; ^{31}P-NMR ($CDCl_3$) : +23.39 & +24.31 ppm.

Fully protected dimer ApsU (12). This compound was prepared in a similar was as for the preparation of compound **11**, employing 150 mg (0.22 mmol) of compound **9**, 149 mg (0.2 mmol) of compound **3** and 356 mg (1.2 mmol) of MSNT. The reaction mixture after the phosphorylation treatment was quenched by addition of water (500 µl),followed by pouring into 0.1 M sodium chloride solution and extracting with dichloromethane (4 x 20 ml). The two diastereomers were separated by short silica gel column chromatography, using the following dichloromethane - ethanol gradient as mobile phase: 0% - 0.5% - 1% - 2% EtOH (1 drop of pyridine / 10 ml solvent). The higher R_f / lower R_f ratio was roughly 60:40 (TLC). Total yield 86 mg (33%) after precipitation from cold hexane. R_f (higher R_f isomer): 0.26 (B); ^{1}H-NMR ($CDCl_3$) : 9.15 (br, 1H) NH ; 8.36 (s, 1H) AH-8 ; 8.08-6.24 (m, 26H) arom. & AH-2, UH-6 ; 6.05 (d, $J_{1',2'}$ = 8.0 Hz, 1H) UH-1′; 5.91 (d, $J_{1',2'}$ = 4.9 Hz,1H) AH-1′; 5.89 (d, $J_{5,6}$ = 8.1 Hz, 1H) UH-5; 5.55-5.32 (m, 3H) AH-2′,UH-2′, UH-3′ ; 4.71-4.28 (m, 7H), AH-3′, AH-4′, AH-5′ & 5′′, UH-4′ , POCH_2CH_2CN; 3.47 (d, $J_{5',5''}$ = 5.4 Hz, 1H) UH-5′; 3.30 (d, 1H) UH-5′′ ; 2.78 (t, J = 6.1 Hz, 2H) -$POCH_2CH_2CN$; 2.48 (s, 3H) -COPhCH_3; 2.10, 2.06 (2 x s, 2 x 3H) 2 x -$COCH_3$; ^{31}P-NMR ($CDCl_3$): +27.36 ppm ; R_f (lower R_f isomer): 0.23 (B); ^{1}H-NMR ($CDCl_3$): 9.05 (br, 1H) NH; 8.39 (s, 1H) AH-8 ; 8.08-6.24 (m, 31H) arom. & AH-2, UH-6 ; 6.02 (d, $J_{1',2'}$ = 7.6 Hz, 1H) UH-1′; 5.86 (d, $J_{5,6}$ = 7.8 Hz, 1H) UH-5 ; 5.87 (d, $J_{1',2'}$ = 5.1 Hz, 1H) AH-1′ ; 5.56-5.29 (m, 3H) AH-2′ , UH-2′, UH-3′; 4.73 (m, 1H) AH-3′ ; 4.55-4.24 (m, 6H) AH-4′, AH-5′ & 5′′, UH-4′, -POCH_2CH_2CN;

3.39 (m, 2H) UH-5′ & 5′′; 2.77 (t, J = 6.3 Hz, 2H) -POCH$_2$*CH$_2$*CN; 2.48 (s, 3H) -COPh*CH$_3$*; 2.06 (s, 6H) 2 x -COCH$_3$; ^{31}P-NMR ($CDCl_3$): +26.57 ppm.

Fully protected dimer ApsU (13). This compound was prepared in the same manner as for the preparation of compound **12**, employing 124 mg (0.183 mmol) of compound **9**, 95 mg (0.157 mmol) of N-6-benzoyl-2′-O-(t-butyldimethylsilyl)-5′-O-toluoyladenosine[44] and 232 mg (0.79 mmol) of MSNT. Isolation of the two diastereomers was achieved by short silica gel column chromatography,using dichloromethane - ethanol (99:1 v/v) and (98:2 v/v) as sequence of mobile phases. The higher and lower R_f ratio was roughly 1 : 1 (TLC). Total yield 94 mg (52%); R_f (higher R_f isomer): 0.29 (B) ; ^{1}H-NMR ($CDCl_3$): 9.14 (br, 1H) NH ; 8.74 (s, 1H) AH-8 ; 8.20 (s, 1H) AH-2; 8.08-7.22 (m, 15H) arom. & UH-6 ; 6.07 (d, $J_{1',2'}$ = 5.1 Hz, 1H) UH-1′; 5.90 (d, $J_{1',2'}$ = 4.9 Hz, 1H) AH-1′ ; 5.89 (d, J = 8.1 Hz, 1H) UH-5; 5.53-5.21 (m, 3H) AH-2′, UH-2′, UH-3′; 4.90-4.57 (m, 3H) AH-3′, AH-4′, UH-4′; 4.47-4.25 (m, 4H) AH-5′ & 5′′, -POCH$_2$CH$_2$CN; 3.44 (d, $J_{5',5''}$ = 5.1 Hz, 1H) UH-5′; 3.26 (d, 1H) UH-5′′; 2.80 (t, J = 5.9 Hz, 2H) -POCH$_2$*CH$_2$*CN; 2.41 (s, 3H) -COPh*CH$_3$* ; 2.09, 2.06 (2 x s, 2 x 3H) 2 x -COCH$_3$; 0.80 (s, 9H) t-butylSi ; 0.05 (s, 3H) CH$_3$Si ; -0.16 (s, 3H) CH$_3$Si; ^{31}P-NMR ($CDCl_3$): +28.34 ppm. R_f (lower R_f isomer): 0.25 (B) ; ^{1}H-NMR ($CDCl_3$): 9.14 (br, 1H) NH; 8.73 (s, 1H) AH-8; 8.21 (s, 1H) AH-2 ; 8.07-7.20 (m, 15H) arom. & UH-6; 6.06 (d, $J_{1',2'}$ = 4.4 Hz,1H) UH-1′ ; 5.90 (d, J = 8.3 Hz, 1H) UH-5; 5.86 (d, $J_{1',2'}$ = 4.6 Hz, 1H) AH-1′; 5.54-5.27 (m, 3H) AH-2′, UH-2′, UH-3′; 4.92-4.59 (m, 3H) AH-3′, AH-4′, UH-4′; 4.47-4.23 (m, 4H) AH-5′ & 5′′, PO*CH$_2$*CH$_2$CN; 3.38 (m, 2H) UH-5′ & 5′′; 2.80 (t, J = 6.4 Hz, 2H) -POCH$_2$*CH$_2$*CN; 2.39 (s, 3H) -COPh*CH$_3$* ; 2.10, 2.06 (2 x s, 2 x 3H) 2 x COCH$_3$; 0.81 (s, 9H) t-butylSi; 0.05 (s, 3H) CH$_3$Si ; -0.18 (s, 3H) CH$_3$Si ; ^{31}P-NMR ($CDCl_3$): +28.44 ppm.

Fully protected dimer d(TpsT) (14). This compound was prepared in the same manner as for the preparation of compound **12**, employing 95 mg (0.142 mmol) of compound **10**, 84 mg (0.164 mmol) of 5′-O-(4-methoxytrityl)thymidine and 221 mg (0.745 mmol) of MSNT.Short silica gel column chromatography provided the title compound as an inseparable mixture of the two diastereomers.Yield 63% (95 mg), after precipitation from cold hexane. R_f: 0.23 (B); ^{1}H-NMR (R_p & S_p, $CDCl_3$) : 7.97-6.79 (m, 21H) arom. & 2 x H-6 ; 6.44, 6.21 (m, t, 2 x 1H) 2 x H-1′ ; 5.42-5.08 (m, 2H) 2 x H-3′ ; 4.41-4.11 (m, $^3J_{HCOP}$ = 8.1 Hz, 4H) 2 x H-4′ & -PO*CH$_2$*CH$_2$CN ; 3.79 (s, 3H) -OCH$_3$; 3.57-3.14 (m, 4H) 2 x (H-5′ & 5′′) ; 2.77, 2.70 (2 x t, J = 5.6 Hz, 2H) -POCH$_2$*CH$_2$*CN ; 2.56-2.29 (m, 4H) 2 x (H-2′ & 2′′) ; 2.08, 2.06 (2 x s, 2 x 3H) -COCH$_3$; 1.96 (s, 3H) 5-CH$_3$ of -psdT ; 1.45 (s, 3H) 5-CH$_3$ of dTp- ; ^{31}P-NMR ($CDCl_3$): +27.71 ppm.

Fluoride treatment of the fully protected dimer (11). **11** (155 mg, 113 µmol) was dissolved in 5 ml of a mixture of tetrahydrofuran-pyridine-water (8:1:1 v/v/v). To this solution, tetra n-butylammonium fluoride trihydrate (72.5 mg, 230 µmol) was added and stirred for 1h at 20 °C. The volatile matters were removed by evaporation *in vacuo* and co-evaporated a couple of times with toluene. The residue was subjected to purification by short silica gel column chromatography, using a sequence of mobile phases ranging from dichloromethane-hexane (50:50 v/v) to dichloromethane- ethanol (90:10 v/v). Pyridine was added to all mixtures (1dr / 10 ml). The appropriate fractions corresponding to each product were pooled. Those products that did not separate from each other during this purification step, were separated by preparative thin layer chromatography,using solvent B as mobile phase. All the compounds were co-evaporated with toluene and carbon tetrachloride and further dried over phosphorus pentoxide. Following compounds were characterized: **6-N-benzoyl-2′-O-pixyl-5′-O-toluoyladenosine-3′-phosphoromonofluoridate n-tetrabutyl ammonium salt (20).** Yield 89 mg (74%). R_f: 0.53 (D) ; UV (95% EtOH): λ_{max} 226, 235 (sh.), 243 (sh.), 282 and 291(sh.) nm ; ^{1}H-NMR ($CDCl_3$, 270 MHz) : 9.05 (br, 1H) NH ; 8.04 (s, 1H) H-8 ; 7.67 (s, 1H) H-2; 7.93-6.06 (m, 22H) arom.; 6.00 (d, $J_{1',2'}$ = 6.3 Hz, 1H) H-1′ ; 5.04 (m, 1H) H-2′; 4.66 (br, 1H) H-4′ ; 4.72 (dd, $J_{5',5''}$ = 12.0 Hz, $J_{5',4'}$ = 4.3 Hz, 1H) H-5′ ; 4.69 (dd, 1H) H-5′′ ; 4.08 (t, $J_{3',4'}$ = 7.2 Hz, 1H) H-3′ ; 3.14-3.00 (m, 8H) $^+$N(*CH$_2$*CH$_2$CH$_2$CH$_3$)$_4$; 2.37 (s, 3H) -COPh*CH$_3$* ; 1.60-0.87 (m, 28H) $^+$N(CH$_2$*CH$_2$CH$_2$CH$_3$)$_4$; ^{31}P-NMR ($CDCl_3$) : +5.62 & -20.22 ppm ; d, J_{PP} = 937 Hz. **6-N-benzoyl-2′-O-pixyl-5′-O-toluoyladenosine (3).** Yield 19 mg (23%) ; R_f: 0.31 (B) ; ^{1}H-NMR ($CDCl_3$) : 8.31 (s, 1H) H-8 ; 8.02-6.81 (m, 23H) arom. & H-2 ; 5.95 (d, $J_{1',2'}$ = 7.3 Hz, 1H) H-1′ ; 4.90 (m, 1H) H-2′; 4.56-4.19 (m, 3H) H-4′, H-5′ & 5′′ ; 3.38 (d, $J_{3',2'}$ = 5.2 Hz, 1H) H-3′ ; 2.86 (s, 3H) -COPh*CH$_3$*. **3-N-benzoyl-2′,3′-di-O-acetyl-5′-deoxy-5′-S-benzoyluridine (16).** Yield 14 mg (22.5%). R_f: 0.72 (B) ; UV (95% EtOH): λ_{max} 247 and 256 (sh.) nm ; ^{1}H-NMR ($CDCl_3$) : 8.04-7.20 (m, 11H) arom. & H-6 ; 5.95 (d, $J_{1',2'}$ = 4.9 Hz, 1H) H-1′ ; 5.83 (d, J = 8.1 Hz, 1H) H-5 ; 5.46-5.18 (m, 2H) H-2′, H-3′; 4.39 (m, 1H) H-4′ ; 3.54 (d, $J_{5',5''}$ = 5.4 Hz, 2H) H-5′ & 5′′ ; 2.12, 2.05 (2 x s, 2 x 3H) 2 x -COCH$_3$; MS (FAB$^-$): calc. for (M-Bz)$^-$ 447.0862, found 447.0869. **2′,3′-di-O-acetyl-5′-deoxy-5′-S-benzoyluridine (17).** Yield 5 mg (10%). R_f: 0.31 (B) ; UV (95% EtOH): λ_{max} 247 and 259 (sh.) nm ; ^{1}H-NMR ($CDCl_3$) : 8.68 (br, 1H) NH; 8.01-7.31 (m, 6H) arom. & H-6 ; 5.92 (d, $J_{1',2'}$ = 4.9 Hz, 1H) H-1′; 5.72 (d, J = 8.3 Hz, 1H) H-5 ; 5.46-5.18 (m, 2H) H-2′, H-3′; 4.37 (m, 1H) H-4′; 3.53 (d, $J_{5',5''}$ = 5.6 Hz, 2H) H-5′ & 5′′; 2.13, 2.09 (2 x s, 2 x

3H) 2 x -$COCH_3$; MS (FAB^-): calc. for $(M-H)^-$ 447.0862, found 447.0869. **Bis(5′-deoxy-3-N-benzoyl-2′,3′-di-O-acetyl-5′-uridyl) disulfide (18).** Yield 7 mg (14%). R_f: 0.43 (B) ; UV(95% EtOH): λ_{max} 253 nm ; ^{1}H-NMR ($CDCl_3$) : 8.02-7.44 (m, 12H) arom. & 2 x H-6 ; 5.92 (d, J = 8.1 Hz, 2H) 2 x H-5 ; 5.95 (m, 2H) 2 x H-1′; 5.55-5.39 (m, 4H) 2 x (H-2′, H-3′) ; 4.37 (m, 2H) 2 x H-4′; 3.30 (dd, $J_{5',5''}$ = 14.4 Hz, $J_{5',4'}$ = 5.4 Hz, 2 x 1H) 2 x H-5′; 3.08 (dd, $J_{5'',4'}$ = 5.6 Hz, 2 x 1H) 2 x H-5′′; 2.12, 2.08 (2 x s, 4 x 3H) 4 x -$COCH_3$; MS (FAB^+): calc. for M^+ 894.1724, found 894.1774. **(5′-deoxy-3-N-benzoyl-2′,3′-di-O-acetyl-5′-uridyl)-(5′-deoxy-2′,3′-di-O-acetyl-5′-uridyl) disulfide (19).** Yield 16 mg (36%). R_f: 0.30 (B) ; UV(95% EtOH): λ_{max} 253 nm ; ^{1}H-NMR ($CDCl_3$) : 8.66 (br, 1H) NH ; 8.01-7.44 (m, 7H) arom. & 2 x H-6 ; 6.01-5.75 (m, 4H) 2 x (H-1′, H-5); 5.52-5.39 (m, 4H) 2 x (H-2′, H-3′) ; 4.37 (m, 2H) 2 x H-4′; 3.31 (dd, $J_{5',5''}$ = 15.0 Hz, $J_{5',4'}$ = 5.1 Hz, 2 x 1H) 2 x H-5′ ; 3.07 (dd, $J_{5'',4'}$ = 5.9 Hz, 2 x 1H) 2 x H-5′′ ; 2.13, 2.11, 2,10, 2.07 (4 x s, 4 x 3H) 4 x -$COCH_3$; MS (FAB^+): calc. for M^+ 790.1462, found 790.1475.

Acid hydrolysis of the fully protected adenosine 3′-O-phosphoromonofluoridate n-tetrabutylammonium salt (20). (a) **20** (57 mg, 54 μmol) was dissolved in 80% aqueous acetic acid (1 ml) and stirred for 30 min at 20 °C. The reaction mixture was evaporated *in vacuo* and the residue was then once co-evaporated with 1.5 M Et_3N^+ HCO_3^-, pH 8.5 (1 ml), followed by co-evaporation with water (1x1 ml). ^{31}P-NMR (CD_3OD) of the residue gave the following absorptions: +19.42 ppm (13%) and +1.12, +0.34 ppm (together 87%). The residue was subjected to preparative thin layer chromatography using solvent F as mobile phase. (b). The acid hydrolysis (a) was repeated, but this time the reaction mixture was stirred for 12 h at 20 °C. ^{31}P-NMR (CD_3OD) of the resulting residue showed now only the absorptions at +1.12 and +0.34 ppm (3:2 ratio).

6-N-benzoyl-5′-O-toluoyl-2′,3′-O-cyclic adenylic acid n-tetrabutylammonium salt (21). Yield 5 mg (11%), isolated from acid hydrolysis (a). R_f: 0.45 (F) ; UV (95% EtOH) : λ_{max} 237 and 278 nm ; ^{1}H-NMR (CD_3OD, 270 MHz) : 8.67 (s, 1H) H-8 ; 8.61 (s, 1H) H-2; 8.18-7.30 (m, 9H) arom.; 6.47 (d, $J_{1',2'}$ = 3.1 Hz, 1H) H-1′; 5.81 (ddd, $J_{2',3'}$ = 6.9 Hz, $^3J_{HCOP}$ = 7.5 Hz, 1H) H-2′; 5.43 (m, $J_{3',4'}$ = 4.9 Hz, $^3J_{HCOP}$ = 11.4 Hz, 1H) H-3′; 4.79 (m, 1H) H-4′ ; 4.66-4.59 (m, 2H) H-5′ & 5′′ ; 3.37-3.26 (m, 8H) $^+N(CH_2CH_2CH_2CH_3)_4$; 2.45 (s, 3H) -COPhCH_3; ; 1.82-1.39 (m, 16H) $^+N(CH_2CH_2CH_2CH_3)_4$; 1.12 (t, 12 H) $^+N(CH_2CH_2CH_2CH_3)_4$ ^{31}P-NMR (CD_3OD) : +19.4 ppm.

Isomeric mixture of 2′- and 3′-(6-N-benzoyl-5′-O-toluoyl)adenylic acid n-tetrabutylammonium salt (22) and (23). Yield 29 mg (66%),isolated from acid hydrolysis (b). R_f: 0.28 (F) ; UV (95% EtOH): λ_{max} 238 and 280 nm ; ^{1}H-NMR (DMSO-d_6, 270 MHz) : 8.69 (m, 2H) H-8, H-2; 8.15-7.42 (m, 9H) arom.; 6.24 (d, $J_{1',2'}$ = 4.9 Hz, 1H) H-1′ of 2′-phosphate isomer ; 6.16 (d, $J_{1',2'}$ = 6.6 Hz, 1H) H-1′ of 3′-phosphate isomer (2:3 ratio); 5.25 (q, $J_{2',3'}$ = 5.3 Hz, $^3J_{HCOP}$ = 5.1 Hz, 1H) H-2′ of 2′-phosphate isomer ; 5.01 (dd, $J_{2',3'}$ = 5.1 Hz, 1H) H-2′ of 3′-phosphate isomer ; 4.86 (m, $J_{3',4'}$ = 3.2 Hz, $^3J_{HCOP}$ = 8.5 Hz, 1H) H-3′ of 3′-phosphate isomer ; 4.70-4.35 (m) H-4′and H-5′ & 5′′ of both isomers ; 3.30-3.20 (m, 8H) $^+N(CH_2CH_2CH_2CH_3)_4$; 2.48 (s, 3H) -COPhCH_3; 1.76-1.30 (m, 16H) $^+N(CH_2CH_2CH_2CH_3)_4$; 1.03 (t, 12H) $^+N(CH_2CH_2CH_2CH_3)_4$; ^{31}P-NMR (DMSO-d_6) : +1.12 & +0.34 ppm (3:2 ratio).

Partial deprotection and decomposition of the fully protected dimer ApsU (12). Compound **12** (63.4 mg, 48.5 μmol) was dissolved in dry pyridine (500 μl), followed by addition of 101 μl (728 μmol) dry triethylamine. After stirring for 4 h at 20 °C, the reaction mixture was evaporated and co-evaporated with toluene *in vacuo* .The crude product **37** [(^{31}P-NMR (pyridine-d_5): +14.6 ppm as the sole absorption; ^{1}H-NMR (pyridine-d_5) : 8.62 (s, 1H) AH-8; 8.47-6.59 (m, 23 H) arom. & UH-6 ; 8.34 (s, 1H) AH-2 ; 6.19-6.04 (m, $J_{5,6}$ = 8.0 Hz, 3H) AH-1′, UH-1′, UH-5; 3.84 (m, 2H) UH-5′, 5"; 3.07 (q, 6H) -CH_2 of triethylammonium; 2.31 (s, 3H) -COPhCH_3, 2.07, 1.94 (2 x s, 2 x 3H) 2 x -COCH_3 ; 1.27 (t, 9H) CH_3 of triethylammonium] was then dissolved in 80% aq. acetic acid (4 ml) and stirred for 30 min at 20 °C. The volatile matters were removed by evaporation *in vacuo* and the residue was co-evaporated with 1.5 M Et_3N^+ HCO_3^-, pH 8.5 (1 x 4 ml) and water (1 x 4 ml). R_f (dimer **39**) : 0.69 (E) ; ^{31}P-NMR ($CDCl_3$) : +17.26 ppm, as the sole absorption. The crude mixture of **20** was dissolved in acetonitrile - water (5:1 v/v, 3 ml). Silica gel (20 mg) was added and the suspension (pH ~ 5) was stirred for 3 days at 40 °C. The silica was removed by filtration and the filtrate evaporated *in vacuo* . After co-evaporation with toluene, the residue was subjected to preparative thin layer chromatography, using solvent G as mobile phase. All the main bands were collected separately and the organic material obtained from the front band was again run on a preparative thin layer plate using solvent B as mobile phase. After two runs in the same solvent, two main bands were collected separately. Following products were isolated and characterized: **6-N-benzoyl-5′-O-toluoyl-2′,3′-O-cyclic**

adenylic acid (40). Yield 20 mg (74.8%) ; R_f: 0.34 (G) ; UV (95% EtOH) : λ_{max} 238 nm and 278 nm; ^{1}H-NMR (D_2O, 270 MHz) : 8.45 (s, 1H) H-8 ; 8.30 (s, 1H) H-2 ; 7.90-7.54 (m, 5H) benzoyl- ; 7.58, 7.79 (2 x d, 2 x 2H) toluoyl- ; 6.33 (d, $J_{1',2'}$ = 2.0 Hz, 1H) H-1′ ; 5.95 (m, $J_{2',3'}$ = 6.0 Hz, $^3J_{HCOP}$ = 9.8 Hz, 1H) H-2′ ; 5.46 (m, $J_{3',4'}$ = 2.3 Hz, $^3J_{HCOP}$ = 8.0 Hz, 1H) H-3′ ; 4.98 (m, 1H) H-4′ ; 4.89 (dd, $J_{5',5''}$ = 12.8 Hz, 1H) H-5′ ; 4.34 (dd, $J_{4',5''}$ = 2.2 Hz, 1H) H-5′′ ; 2.16 (s, 3H) -COPhCH_3.. ^{31}P-NMR (D_2O) : +19.42 ppm. Disulfide **18** : Yield 9 mg (44%) ; Disulfide **19** : Yield 3 mg (16%).

Partial deprotection and decomposion of the fully protected dimer ApsU (13). The cyanoethyl group was removed from **13** in an identical way as for **12**, employing 91 mg (78 µmol) of **13** and 163 µl (1.17 mmol) of dry triethylamine. The crude product after evaporation and co-evaporation with toluene *in vacuo* was purified by short silica gel column chromatography, using dichloromethane - ethanol (93:7 v/v) as mobile phase. Yield of the partial deprotected dimer **38** was 85% (80 mg). R_f: 0.24 (C) ; ^{1}H-NMR ($CDCl_3$ + CD_3OD) : 8.62 (s, 1H) AH-8 ; 8.15 (s, 1H) AH-2 ; 8.10-7.20 (m, 15H) arom. & UH-6; 6.14 (d, $J_{1',2'}$ = 5.9 Hz, 1H) AH-1′; 6.07 (d, $J_{1',2'}$ = 7.8 Hz, 1H) UH-1′ ; 5.89 (d, J = 8.3 Hz, 1H) UH-5 ; 5.63-5.41 (m, 2H) UH-2′,UH-3′; 5.11-4.75 (m, 5H) AH-2′, -3′, -4′, -5′ & 5′′; 4.40 (m, 1H) UH-4′ ; 3.38-2.98 (m & q, 8H) UH-5′ & 5′′, -CH_2 of triethylammonium ; 2.39 (s, 3H) -COPhCH_3 ; 2.09, 1.98 (2 x s, 2 x 3H) 2 x -$COCH_3$; 1.33 (t, 9H) CH_3 of triethylammonium ; 0.72 (s, 9H) t-butylSi ; 0.01 (s, 3H) CH_3Si ; -0.23 (s, 3H) CH_3Si; ^{31}P-NMR ($CDCl_3$): +16.33 ppm. The decyanoethylated dimer **38** (75 mg, 62 µmol) was dissolved in dry tetrahydrofuran (1ml). To this solution, TBAF trihydrate (59 mg, 186 µmol) was added and the reaction mixture was stirred for 12 h at 20 °C. The volatile matters were removed by evaporation *in vacuo* and the excess TBAF was removed by a preparative TLC plate using the solvent G as mobile phase. All the UV absorbing materials were collected, pooled and evaporated, the residue containing **39** was then given the same decomposion treatment as for the dimer **12** .The resulting products were isolated in an identical manner as described for the dimer **12**. Following compounds were isolated and characterized: Cyclic phosphate **21** (25 mg, 58%) ; Undecomposed dimer **39** (10 mg, 16% of the starting amount) ; Disulfide **18** (3 mg, 11%) ; Disulfide **19** (7 mg, 29%).

Fully deprotected dimer d(TpsT) (24). Dimer **14** (32 mg, 30 µmol) was dissolved in dry pyridine (250 µl) and to this solution dry triethylamine (65 µl, 465 µmol) was added. After stirring for 4 h at 20 °C, the solution was evaporated *in vacuo* and the residue [^{31}P-NMR ($CDCl_3$) : +16.70 ppm as the sole absorption] dissolved in 35% aqueous ammonia (4 ml) and stirred for 12 h at 20 °C. The solution was evaporated and co-evaporated with water *in vacuo* and the residue [^{31}P-NMR (D_2O) : +19.30 ppm as the sole absorption] was dissolved in 80% aq. acetic acid (4 ml) and stirred for 6 h at 20 °C. The solution was evaporated and co-evaporated with water (1 x 4 ml) and 1.5 M Et_3N^+ HCO_3^-, pH 8.5 (4 ml) *in vacuo* . The residue [^{31}P-NMR (D_2O) : +19.03 ppm as the sole absorption] was dissolved in water (5 ml) and extracted with diethyl ether (2 x 5 ml). The aqueous phase was concentrated to a small volume and applied to a preparative thin layer plate (200 x 200 x 2 mm) and using the solvent F as mobile phase. The appropriate band was collected, the silica washed with water, which was then evaporated to *in vacuo*. The residue was then chromatographed on a short DEAE-Sephadex column (3.5 x 3cm, HCO_3^- form) using linear gradients of Et_3N^+ HCO_3^-, buffer, pH 7.5 [0.001M (200ml) - 0.005M (200ml), 0.005M (200ml) - 0.05M (200ml)]. The appropriate fractions were pooled and evaporated *in vacuo*, followed by co-evaporations with water. The residue was dissolved in a small volume of distilled water and washed through a Dowex column (1 x 18cm, Na^+ form). The product containing eluate was collected and concentrated to 1 ml and then lyophilized to give 9.1 mg (51%) of the title compound. R_f: 0.30 (G) ; UV (H_2O) : λ_{max} 267 nm (pH 2), 267 nm (pH 7) and 267 nm (pH 13) ; ^{1}H-NMR (D_2O, 270 MHz) : 7.57 (s, 1H) H-6 of -psdT ; 7.50 (s,1H) H-6 of dTp- ; 6.21 (dd, $J_{1',2'}$ = 7.5 Hz, $J_{1',2''}$ = 6.3 Hz, 1H) H-1′ of dTp- ; 6.19 (t, $J_{1',2'}$ & $J_{1',2''}$ = 6.7 Hz, 1H) H-1′ of -psdT ; 4.81 (m, $J_{2',3'}$ = 6.4 Hz, $J_{2'',3'}$ = 3.1 Hz, $J_{3',4'}$ = 3.0 Hz, 1H) H-3′ of dTp- ; 4.39 (m, $J_{2',3'}$ = 6.7 Hz, $J_{2'',3'}$ = 4.6 Hz, $J_{3',4'}$ = 4.4 Hz,1H) H-3′of -psdT ; 4.16 (m, $J_{4',5'}$ = 3.9 Hz, $J_{4',5''}$ = 4.7 Hz, 1H) H-4′ of dTp- ; 4.08 (m, $J_{4',5'}$ = 4.7 Hz, $J_{4',5''}$ = 6.8 Hz, 1H) H-4′ of -psdT ; 3.77 (dd, $J_{5',5''}$ = 12.4 Hz, 1H) H-5′of dTp- ; 3.71 (dd, 1H) H-5′′of dTp- ; 3.10 (m, $J_{5',5''}$ = 13.6 Hz, $^3J_{H\text{-}5'CSP}$ = 11.3 Hz, 1H) H-5′of -psdT ; 2.98 (m, $^3J_{H\text{-}5''CSP}$ = 11.6 Hz, 1H) H-5′′ of -psdT ; 2.56 (m, $J_{2',2''}$ = 14.3 Hz, 1H) H-2′′ of dTp- ; 2.28 (m, $J_{2',2''}$ = 14.2 Hz, 1H) H-2′′ of -psdT ; 2.32 (m, 1H) H-2′ of -spdT ; 2.36 (m, 1H) H-2′′ of dTp- ; 1.83 (s, 3H) 5-CH_3 of -psdT ; 1.82 (s, 3H) 5-CH_3 of dTp- ; ^{31}P-NMR (D_2O) : +19.12ppm.

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