Pergamon

Tetrahedron Vol. 51, No. 10, pp. 2991-3014, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00031-3

The Synthesis and Reactivity of New 2-(*N*,*N*-Diisopropylamino)-3-Methylsulfonyl-1,3,2-Benzoxazaphospholes. The Utility of the 5-Chloro analogue in the One-Pot Synthesis of Oligothiophosphates: [Ap_SppA, Ap_SpppA, ppp5'A2'p_S5'A, m^7Gp_SppA, Ap_Spppp, Ap_Spp]

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Abstract: *The synthesis of 2-(N,N-diisopropylamino)-2,3-dihydro-3-methylsulfonyl-1,3,2-benzoxazaphospholes* ***22***, ***23*** *and* ***24*** *is reported. Their reactivities have been investigated using a variety of acid catalyst under conditions normally employed in phosphoramidite chemistry for oligonucleotide synthesis. The rate (k) of activation of* ***22***, ***23*** *and* ***24*** *by acid catalysis with N-methylanilinium hydrochloride (MAC) to their protonated species* ***25A***, ***26A*** *and* ***27A***, *respectively, (Scheme 2) has been estimated to be 6.813 x 10^{-7} mol^{-1} min^{-1}, 1.237 x 10^{-6} mol^{-1} min^{-1} and 1.972 x 10^{-7} mol^{-1} min^{-1} at 18°C with a ratio of the rates as 0.56 : 1 : 0.16. The 5-chlorobenzoxazaphosphole* ***23*** *selectively activated by MAC gave the intermediate 2-(N-methylanilinium)-5-chlorobenzoxazaphosphole* ***26A*** *(~90% by NMR), which was then reacted with alcohols (nucleosides) to generate the reactive 2-alkoxybenzoxazaphosphole* ***30*** *(Scheme 4) or* ***33-35*** *(Scheme 5) (~70% by NMR). We have then shown that* ***33-35*** *(Scheme 5) react with binucleophilic reagents, ADP, ATP or pyrophosphate, to generate the corresponding P^1-alkoxycyclometatriphosphite intermediate* ***36***, ***37***, ***50***, ***52*** *or* ***54****. These intermediates were then sulfurised to form the P^1-alkoxy-1-thiocyclotriphosphate intermediates* ***38***, ***39***, ***51***, ***53*** *and* ***55****, which were ring-opened by hydrolysis. Thus these steps constituted a one-pot multicomponent reaction (MCR) leading to the synthesis of R_P and S_P mixtures of each of the mono-thioanalogues of naturally-occurring oligophosphates: Ap_SppA (****43****) (10%), Ap_SpppA (****45****) (19%), ppp5'A2'p_S5'A (****46****) (24%), the cap structure m^7Gp_SppA (****47****) (3%), Ap_Spppp (****42****) (23%), Ap_Spp (****41****) (33%) and Ap_Sp (****40****) (7%). The reaction of putative* ***34*** *and ADP gave the desired* ***43*** *(10%) along with pp5'A2'p_S5'A (****44****) (5%). The reaction sequences from* ***34*** *and ATP gave* ***45*** *(19%) and ppp5'A2'p_S5'A (****46****) (24%). The proposed reaction mechanism for the synthesis of* ***43***, ***45*** *and* ***47*** *proceeds via the corresponding (dinucleoside 5')-cyclometatriphosphite intermediates* ***50***, ***54***, ***52*** *and the (dinucleoside 5')-1-thiocyclotriphosphate intermediates* ***51***, ***55***, ***53****. The existence of these cyclic P(III) and P(V) intermediates were supported by ^{31}P- and ^1H-NMR spectroscopy. We here also demonstrate that 5-chlorobenzoxazaphosphole* ***23*** *can be used to synthesise a protected ribonucleoside 2',3'-cyclic phosphorothioate block* ***28****, a protected ribonucleoside 3'-(O-(4-chloro-2-mesylsulfonamido)phenyl phosphorothioate diester block* ***29*** *and bis(2-deoxyadenosine-5')-thymidine-3'-monophosphate* ***32****. The correct coupling of the nucleoside residues to the oligophosphate chain in* ***43***, ***45*** *&* ***47*** *has unequivocally been assessed by 2D ^{31}P-^{31}P and ^1H-^{31}P correlation spectroscopy.*

Several acyclic aliphatic phosphitylating agents **1** - **3** (Fig. 1) have been successfully used in the synthesis of oligonucleotides[1, 2]. Multifunctional heterocyclic five membered P(V) and five and six membered P(III) phosphorylating or phosphitylating reagents have also received increased attention in their application to the synthesis of phosphomono-, di- and triesters of biomolecules[3]. The five membered cyclic P(V) acyl phosphates (**4**) (CAP)[3], cyclic enediol phosphates (**5**) (CEP)[3] and ethylenephosphoromonochloridate[3] (**6**) (Fig. 1) have been applied to the synthesis of phospholipids and phosphatidyl choline derivatives[3]. In an early

report[4], *o*-phenylenephosphorochloridate (**7**) (Fig. 1) was used for the preparation of nucleoside monophosphates. Phospholipids and phosphatidyl choline derivatives have also been synthesised by using cyclic five membered P(III) reagents such as 2-diethylamino-3-methyl-1,3,2-oxazaphospholidine[3] (**8**), 2-diethylamino-1,3,2-dioxaphospholane[3](**9**), 2-chloro-3-methyl-1,3,2-oxazaphospholidine[3] (**10**) (Fig. 1). Oligonucleotide analogues have been prepared by using the cyclic five membered P(III) reagents: 2-diisopropylamino-1,3,2-thiaoxaphospholidine[3] (**11**) and 2-methoxy-3-(trifluoracetyl)-1,3,2-oxazaphospholidine[3] (**12**) (Fig. 1). The cyclic six membered P(III) reagent 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (salicylphosphorochloridite) (**13**) (Fig. 1) has been used for preparation of nucleoside 3'-H-phosphonates[5] and nucleoside 5'-triphosphates[6], 1-thiotriphosphates[6] and 1,1- and 1,3-dithiotriphosphates[7]. Typical for these cyclic reagents is that they can be made to undergo two successive nucleophilic displacement reactions at the phosphorous centre by two different alcohols under two distinctly different conditions to give the ring-opened acyclic phosphotriester, which then can be conveniently converted to the required phosphodiester.

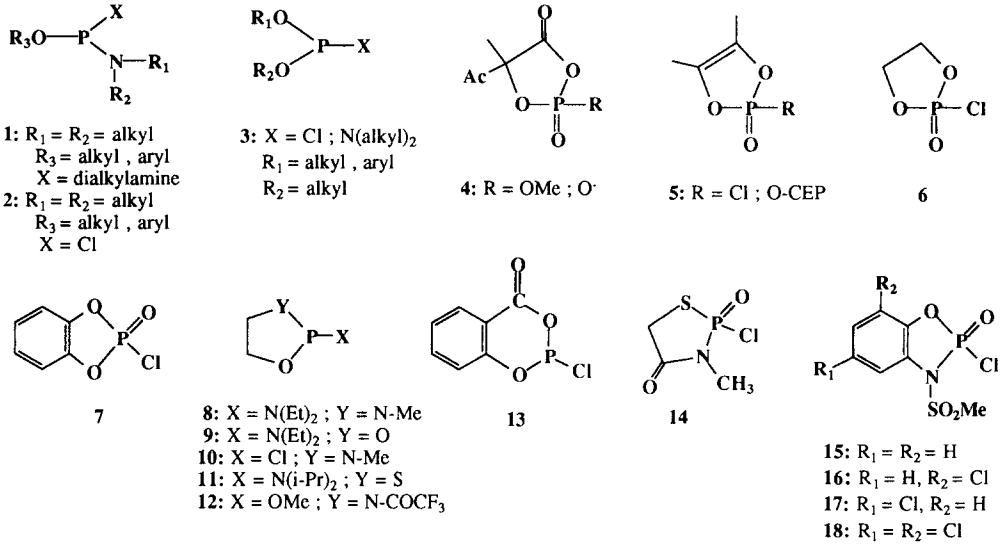


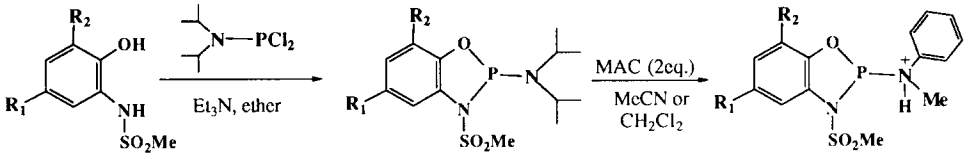
Figure 1

The CAP- and CEP derivatives were too reactive for regiospecific generation of phosphodiester bonds in oligonucleotide synthesis. Ramirez *et al*[8] and Ugi *et al*[9] have independently synthesised a number of analogues of the CAP and CEP derivatives and applied them to oligonucleotide synthesis with limited success. Ugi *et al*[10] demonstrated the use of 2-chloro-2,4-dioxo-3-methyl-tetrahydro-1,3,2-thiazaphosphole (**14**) (Fig. 1) by the synthesis of dithymidine(3'→5')-S-(N-methylcarbamoylmethyl)phosphorothioate through a two-step one-pot phosphorylation. They subsequently designed and synthesised the aromatic 2-chloro-2,3-dihydro-3-(methylsulfonyl)-1,3,2-benzoxazaphosphole-2-oxide[11,12] (**15**) (Fig.1) and later on, the corresponding P(V) reagents with chlorine substituents in the aromatic ring[12,13] (**16 - 18**, Fig.1). These reagents were used for phosphorylation of simple alkanols and hydroxypeptides in a similar way as for **14**.

We here report the synthesis of 2-(*N*,*N*-diisopropylamino)-2,3-dihydro-3-methylsulfonyl-1,3,2-benzoxazaphosphole **22**, its 5-chloro derivative **23** and 5,7-dichloro derivative **24**, and subsequently show the utilisation of **23** as an convenient reagent for the synthesis of P^1,P^3-(diadenosine 5')-1-thiotriphosphate [Ap_SppA (**43**)], P^1,P^4-(diadenosine 5')-1-thiotetraphosphates [Ap_SpppA (**45**)], the cap structure P^1-(*N*-7-methylguanosine 5')-P^3-(adenosine 5')-1-thiotriphosphate [m^7Gp_SppA (**47**)] and adenosine 5'-(1-thiodi-, tri- and pentaphosphates) [Ap_Sp (**40**), Ap_Spp (**41**) and Ap_Spppp (**42**)] (Scheme 5). The corresponding oxygen analogues of these oligothiophosphates derivatives are known to be biologically functional[14-24], and it has been shown that some of these thio analogues have considerable application in biochemistry and molecular biology[6, 25, 26]. We have also demonstrated in this work that **23** can be used successfully for the synthesis of derivatives of adenosine 2',3'-cyclic phosphorothioate (**28**), a cytidine 3'-(*O* -(4-chloro-2-mesylamino)phenylphosphorothioate diester block (**29**) and bis(2-deoxyadenosine-5')-thymidine-3'-monophosphate (**32**).

RESULTS AND DISCUSSION

(A) Synthesis of benzoxazaphospholes 22, 23 & 24. The synthesis of *N*-mesylated compounds **19**, **20** and **21** from their corresponding amino phenol derivatives have been recently reported[13]. Upon condensation of *N*-mesylated phenols **19**, **20** & **21** with dichloro-(*N*,*N*-diisopropylamino)phosphine in triethylamine and dry diethyl ether, benzoxazaphospholes **22** ($\delta^{31}P$ = +131.4 ppm), **23** ($\delta^{31}P$ = +135.0 ppm) and **24** ($\delta^{31}P$ = +137.0 ppm) (Scheme 1) were obtained as solids after crystallisation from diethyl ether / petroleum ether mixtures (see experimental).



19: $R_1 = R_2 = H$
20: $R_1 = Cl$, $R_2 = H$
21: $R_1 = Cl$, $R_2 = Cl$

22: $R_1 = R_2 = H$
23: $R_1 = Cl$, $R_2 = H$
24: $R_1 = Cl$, $R_2 = Cl$

25A: $R_1 = R_2 = H$
26A: $R_1 = Cl$, $R_2 = H$
27A: $R_1 = Cl$, $R_2 = Cl$

Scheme 1

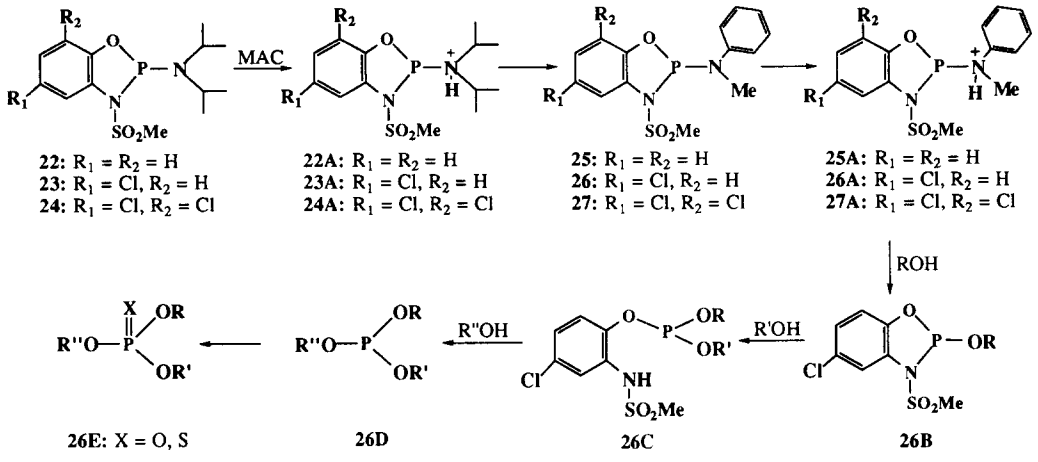
(B) Activation of benzoxazaphospholes 22, 23 and 24 with acid catalysts and reactions with alcohols. Each of the activation experiments on **22**, **23** and **24** were carried out in a NMR-tube under an inert atmosphere of argon and the reactions were followed by ^{31}P-NMR. Attempts to activate benzoxazaphosphole **23** by tetrazole[27,28,29] in MeCN, diisopropylammonium tetrazolide (DIPAT)[30] in CH_2Cl_2 and 5-(4-nitrophenyl)tetrazole (NTP)[31,32] in MeCN were unsuccessful. However, addition of a slight excess of dry ethanol to these reaction mixtures led to a slow consumption (17 h - 72 h) of **23** into triethylphosphite [85-40% (NMR), $\delta^{31}P$ = +138.5 ppm] in a non-specific manner. This is consistent with an earlier published report[33] where it was shown that nucleoside *O*-arylphosphoramidites with an electron-withdrawing

substituent in the *ortho*-position of the aryl ring when activated with tetrazole had very low coupling rates with 5'-hydroxy nucleosides.

The activation of **23** [δ^1H = 2.95 ppm (s) SO_2Me; δ^{31}P = +135.0 ppm] by *N*-methylanilinium hydrochloride (MAC) in MeCN ($t_{99.5} \approx 40$ min) was however more successful, giving the *N*-methylanilinium derivative **26A** [δ^1H = 2.80 ppm (d, $J_{N\text{-}Me,P}$ = 4.6 Hz) *Me*-NH$^+$, 3.10 ppm (s) SO_2Me; δ^{31}P = +126.0 ppm] (Scheme 1) and by-products (δ^{31}P ≈ +2.0 - +6.0 ppm) in a 9 : 1 ratio (NMR). The MAC activation of **23** to **26A** was somewhat slower in CH_2Cl_2 (85% by NMR). A similar experiment with **22** in CH_2Cl_2 gave the corresponding *N*-methylanilinium derivative **25A** [δ^1H = 2.77 ppm (d, $J_{N\text{-}Me,P}$ = 4.0 Hz) *Me*-NH$^+$, δ^{31}P = +123.0 ppm] (Scheme 1) and by-products (δ^{31}P ≈ 2.0 - 6.0 ppm) ($t_{99.5} \approx 15$ min) in a 1 : 1 ratio (NMR). When **24** was similarly activated in MeCN the corresponding *N*-methylanilinium derivative **27A** [δ^1H = 2.84 ppm (d, $J_{N\text{-}Me,P}$ = 5.7 Hz) *Me*-NH$^+$, δ^{31}P = +127.3 ppm] (Scheme 1) was formed ($t_{99.5} \approx 150$ min) together with the by-products (δ^{31}P = ~2.0 - 6.0 ppm) in a 6 : 4 ratio (NMR). The foregoing studies clearly showed that the formation of the desired *N*-methylanilinium species over the by-products upon MAC activation of **22**, **23** and **24** is most favoured in case of the reagent **23**.

The unequivocal evidence that the protonated species **25A**, **26A** and **27A** are indeed formed in the MAC promoted activation of **22**, **23** or **24**, respectively, was proven by first synthesising *N*-methylanilino derivative **25** [δ^1H = 2.85 ppm (d, $J_{N\text{-}Me,P}$ = 4.2 Hz) *Me*-N, δ^{31}P = +123.0 ppm] (Scheme 2) and subjecting it to the MAC activation: thus **25** was dissolved in $CDCl_3$ in a NMR-tube and titrated with MAC (0.1 to 0.8 eq). The proton chemical shift of the *N*-methyl group was found to move upfield to δ^1H = 2.75 ppm (d, $J_{N\text{-}Me,P}$ = 4.0 Hz) compared to the neutral **25**, which was identical to the change of chemical shift and coupling constant found upon addition of MAC to **22**, suggesting that the active protonated species to be *N*-methylanilinium derivative **25A** in both cases. Note that the chemical shift of *N*-Me group in MAC [δ^1H = 3.04 ppm (s)] as expected moved downfield compared to the parent *N*-methylaniline [δ^1H = 2.91 ppm (s)], whereas the *N*-Me group in the protonated species **25A**, **26A** or **27A** moved upfield by almost the same magnitude of $\Delta\delta$, compared to neutral **25**, owing to the unique effect of phosphorous shielding on the covalently bonded *N*-Me group, which is presumably dependant upon the geometry across P-N$^+$ bond as well as on the modes of the delocalization between endocylic phosphorus and *N*-methylanilinium-nitrogen atom[34]. A comparison of the chemical shifts and the coupling constants found for **22** → **25A** with those of the product formed upon addition of MAC to **23** or **26** also suggest that the products formed are indeed the corresponding protonated species **26A** or **27A**, respectively.

When **22**, **23** or **24** was activated with *N*-methylanilinium trichloroacetate[35] and *N*-methylanilinium trifluoroacetate[36,37] under the above conditions, the ratio between the corresponding *N*-methylanilino derivative and the by-product formed (~7 : 3, NMR) was discouraging. When intermediate **26A** was treated with one equivalent of dry methanol, the mono-displaced product, 5-chloro-2-methoxy-2,3-dihydro-3-mesyl-1,3,2-benzoxazaphosphole[12] **33** (Scheme 5), was formed (15 min, ratio 7 : 3; δ^{31}P = +123.0 ppm). This experiment showed that **23**, upon MAC promoted activation, can be used to synthesise the unsymmetrically substituted products. When the same experiment was performed on intermediates **25** and **27**, the ratio between the 2-methoxybenzoxazaphosphole derivative and the by-products were more unfavourable (~4 : 6 by NMR).



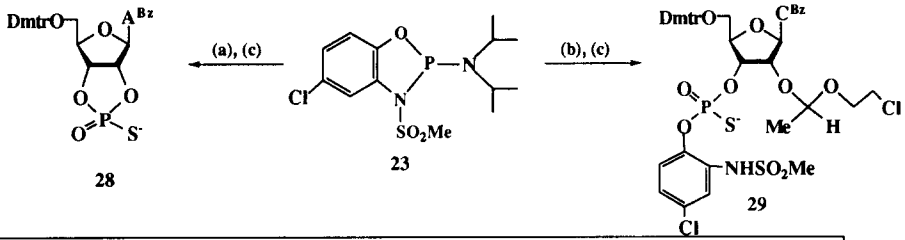
Scheme 2

The MAC activation requires dry handling under argon because of its high hygroscopic nature. Always freshly sublimed material was used. The second order rate constants were determined by ^{1}H-NMR spectroscopy (270 MHz) at 18 °C for the activation of **22**, **23** and **24** by MAC in MeCN to give the corresponding *N*-methylanilinium derivatives **25A**, **26A** and **27A**: k = 6.813 x 10^{-7} mol^{-1} min^{-1} for **22**; k = 1.237 x 10^{-6} mol^{-1} min^{-1} for **23** and k = 1.972 x 10^{-7} mol^{-1} min^{-1} for **24**. Thus, the order of the rate of activation is **23** > **22** > **24** with the ratio of 1 : 0.56 : 0.16. It may be noted that these relative rates of activations is in reversed order to those of the rates found for the ring-opening reactions of the corresponding P(V) reagents[13] with the second alcohol in the second step (i.e. **18** > **15** > **17**, Fig.1).

The chemical reactivity of 2-(*N,N*-diisopropylamino)-2,3-dihydro-3-methylsulfonyl-1,3,2-benzoxazaphosphole **22**, and its chloro derivatives **23** and **24** (Scheme 1) is based upon the relatively more basic character of the exocyclic *N,N*-diisopropylamino group compared to the endocyclic methylsulfonamido function. Scheme 2 shows the proposed mechanism of activation of **23** and its reactions with alcohols. In the first acid catalyzed step, the *N,N*-diisopropylamino nitrogen is protonated (*i.e.* **23A**), which is then displaced by *N*-methylaniline to give **26**, which successively forms the protonated species **26A**. The protonated intermediate **26A** undergoes the first nucleophilic displacement reaction at the phosphorous by an alcohol to give **26B**. The second reaction step involves a second nucleophilic attack at phosphorous by either an alkanol or a phosphate accompanied by a cleavage of the endocyclic N-P bond and opening of the five membered ring, thus giving a double-displaced P(III) intermediate **26C**. The 5-chloro-phenoxy group in **26C** can then be displaced by a third nucleophile to give the intermediate **26D** which can be oxidized to the final P(V) product **26E**. If a binucleophilic reagent such as a 1,2-diol or a pyrophosphate derivative is used in the second step on **26B**, both $MeSO_2N$-P-bond cleavage and displacement of the aryloxy group can take place leading to an overall triple-displaced cyclic P(III) intermediate **26D** (R' and R" are covalently linked), which then easily can be oxidized to the P(V) product **26E** (R' and R" are covalently linked).

(C) Reaction of 5-chloro-benzoxazaphosphole 23 with partially protected ribonucleosides in presence of diisopropylammonium tetrazolide (DIPAT). (i) 6-*N*-benzoyl-5'-*O*-(4,4'-

dimethoxytrityl)adenosine was dissolved in a dry CH_2Cl_2 solution of **23** and DIPAT (Scheme 3). After 38 h, TLC analysis showed that the reaction mixture contained a low-R_f, trityl positive compound, which had been formed quantitatively. Sulfurization and aqueous work up and silica gel chromatography gave the 6-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)adenosine 2',3'-cyclic phosphorothioate **28** (80%) (Scheme 3), which was fully characterized by ^{1}H- and ^{31}P-NMR [δ^{31}P = +74.6 *(Sp)*[6] and +73.0 ppm *(Rp)*[6], *Sp/Rp*, 2 : 1)]. Here we observe that **23** underwent an overall triple-displacement reaction at phosphorous after sulfurisation and aqueous work-up with the aryl group removed to give **28**. (ii) The reaction with 4-*N*-benzoyl-5'-*O*-(4,4'-dimethoxy)trityl-2'-*O*-(1-(2-chloroethoxy)ethyl)cytidine, on the other hand, gave after seven days a complete



28 23 29

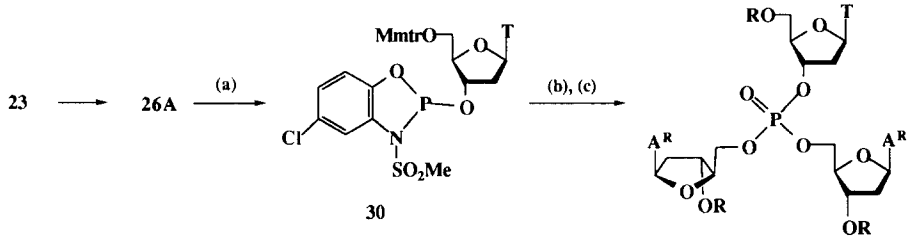
Reagents: (a) Dmtr-A^{Bz}, DIPAT, CH_2Cl_2 ; (b) Dmtr-C^{Bz}(2'-cee), DIPAT, CH_2Cl_2 ; (c) sulfurisation and aq. work-up

Scheme 3

conversion of the starting material into a low-R_f product, as judged by TLC, which upon sulfurisation, aqueous work-up and silica gel chromatography gave 4-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-3'-(*O*-(4-chloro-2-methylsulfonamido)phenyl)phosphorothioate-2'-*O*-(1-(2-chloroethoxyethyl)cytidine **29** (81%) (Scheme 3). Compound **29** was fully characterized by ^{1}H- and ^{31}P-NMR (δ^{31}P = +57.3, +57.0, +55.5 and +55.0 ppm corresponding to four diastereomers originating from the chiral center of the phosphorothioate and the chiral center of the 2'-*O*-protecting group). In this case, owing to the presence of 2'-*O*-protecting group, only one nucleophilic displacement reaction at the phosphorous of **23** took place, which upon sulfurisation and aqueous work-up underwent the second displacement reaction with the concomitant cleavage of the endocyclic P-N bond, leaving the aryloxy group intact. Clearly, the latter conversion of **23** → **29** can be construed as an evidence that that endocyclic P-N bond cleavage is preferred over the endocyclic P-O bond cleavage. Therefore, in the conversion of **23** → **28** , the second of three consecutive nucleophilic displacement reactions involves the cleavage of endocyclic P-N bond whereas the last nucleophilic displacement is promoted by the cleavage of the P-O bond to give **28**.

(D) Reaction of 5-chloro-benzoxazaphosphole 23 with MAC and partially protected 2'-deoxynucleosides for the synthesis of a unsymmetrical trinucleoside monophosphate[38-41]. When 5'-*O*-(4-methoxytrityl)thymidine was dissolved in a MeCN solution of **23** in presence of MAC (Scheme 1), 2-(5'-*O*-(4-methoxytrityl)thymidine-3'-*O*-benzoxazaphosphole derivative **30** was generated (δ^{31}P = +121.5, +120.1 ppm, Scheme 4) in situ. To this mixture an excess of 6-*N*, 3'-*O*-bis(4-methoxytrityl)-2'-deoxyadenosine was added, which led to a disappearance of **30** after 2 h and formation of the trinucleoside phosphitetriester (δ^{31}P = +139.4 ppm), which was oxidized with aqueous iodine to the trinucleoside phosphotriester **31** (16%) (δ^{31}P = -2.0 ppm, Scheme 4). The low yield of **31** was because of accumulation of by-products (δ^{31}P = ~ +2.0 - +6.0 ppm) due to unspecific acid catalyzed hydrolysis in four successive reactions in one-pot. Triester **31** was

deprotected with 80% aq. acetic acid for 4 h to give the fully deprotected trimer **32** (60%) ($\delta^{31}P$ = -2.7 ppm) (see experimentals).

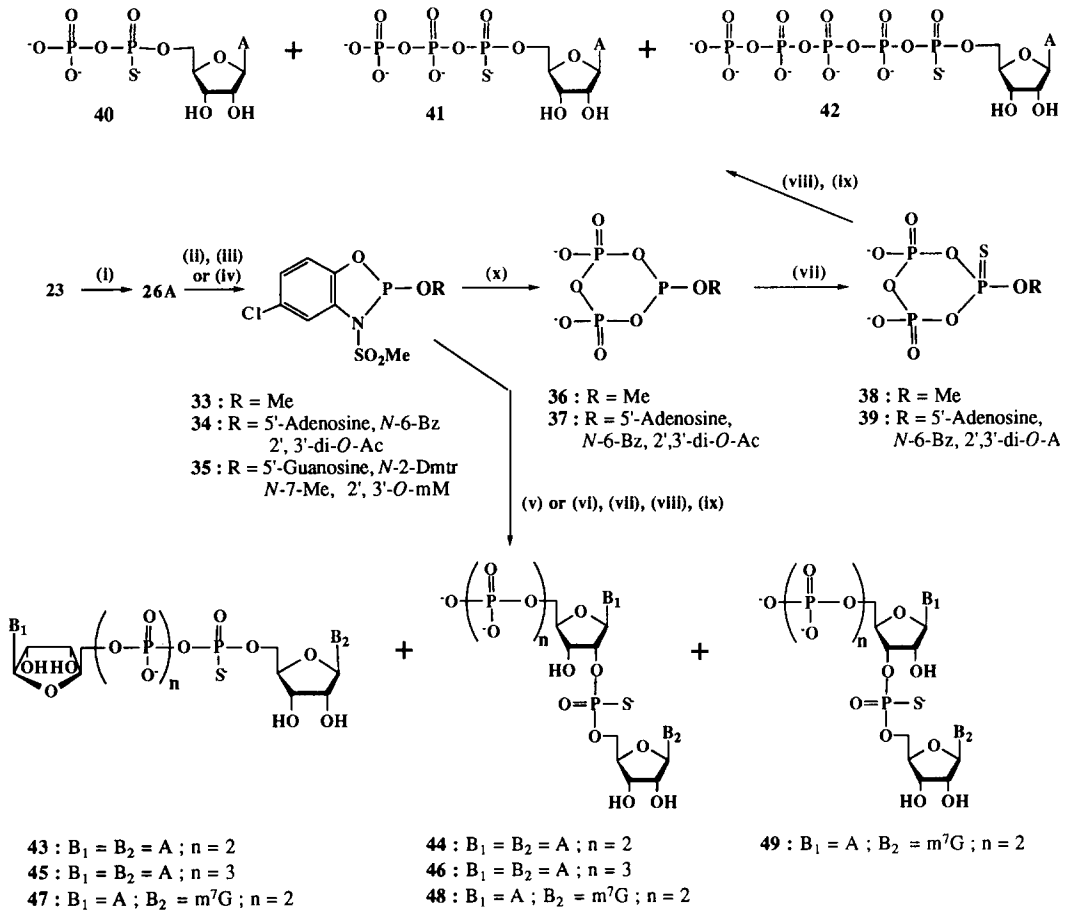


Scheme 4

(E) The five-step-one-pot reaction of 5-chloro-benzoxazaphosphole 23 with a partially protected 5'-hydroxy ribonucleoside in presence of MAC and ADP or ATP to give the thio analogues of ApppA, m^7GpppA and AppppA. 5-Chloro-benzoxazaphosphole **23** has been subsequently successfully used to produce biologically important P^1,P^3-(diribonucleoside 5')-1-thiotriphosphates (Ap_SppA **43** and the cap structure: m^7Gp_SppA **47**) and P^1,P^4-(diribonucleoside 5')-1-tetraphosphates, (Ap_SpppA **45**), which are thioanalogues of naturally occurring ApppA[20], 5'-terminal cap structure m^7GpppA[21,22] and AppppA[20], respectively. The present synthesis thus constitutes the first report of the preparation of oligothiophosphates **43**, **45** and **47** directly by a one-pot reaction from a cyclic P(III) phosphitylating agent. The reaction of 5-chloro-benzoxazaphosphole **23** with a 5'-hydroxy ribonucleoside in the presence of MAC is a common step to give either **34** or **35**, which then reacts with either ADP or ATP to give various oligothiophosphates (Scheme 5).

Reagent **23** was activated with MAC in MeCN to give **26A** ($\delta^{31}P$ = +126.0 ppm, Scheme 5) [step (i)], which was then treated with 6-*N*-benzoyl-2',3'-*O*-diacetyladenosine to produce the cyclic P(III) intermediate **34** ($\delta^{31}P$ = +121.1 & +122.8 ppm) [step (iii)]. The reaction mixture containing intermediate **34** was subsequently added to a dry DMF solution of tri-n-butylamine (4.2 eq) containing either tri-n-butylammonium salt of ADP (1.5 eq) [step (v)] or ATP (1.5 eq) [step (vi)]. Each of the reaction mixture [steps (v & vi)] was then treated with sulfur [step (vii)] followed by hydrolysis [step (viii)] and subsequently by ammonolysis [step (ix)].

(a) Reaction of putative intermediate 34 with ADP (Scheme 5): ^{31}P-NMR of the reaction mixture containing **34** and ADP [step (v)] showed the appearance of down-field absorbances at +154.0 ppm (s), +139.0 ppm (s), +138.0 ppm (m) and +132.0 ppm (m) in a 1.6 : 2.2 : 1 : 1 ratio. Among the upfield absorbances, a new multiplet covering the range from -21.4 to -23.0 ppm was observed. Sulfurisation [step (vii)] caused all the downfield ^{31}P absorptions to shift to +86.5 ppm (s) and +84.0 ppm (s), +57.0 ppm (s), and also a triplet centered at +43.8 ppm in a 2 : 3 : 1: 1 ratio was formed. ^{31}P-NMR showed that hydrolysis [step (viii)] led to the disappearance of the +86.5 and +84.0 ppm absorbances to a set of closely grouped singlets at +57.0 ppm. The +43.8 ppm absorbance however remained intact. The ratio between these two latter groups of absorbances was 1.5 : 1.0. Ammonolysis [step (ix)] and then separation of the reaction mixture on DEAE-



Reagents: (i) MAC in MeCN or CH_2Cl_2 ; (ii) MeOH; (iii) 2',3'-Ac_2-A^{Bz}, MeCN; (iv) 2',3'-mM-m^7G^{Dmtr}, MeCN ; (v) ADP in DMF; (vi) ATP in DMF; (vii) S_8; (viii) H_2O; (ix) NH_3 or H^+; (x) $P_2O_7^{4-}$ in DMF.

Scheme 5

Sephadex A-25 column, followed by further purification by semi-preparative RP-HPLC chromatography resulted in separation of four products (see experimental): two of these products corresponded to the desired *Sp* and *Rp* isomers of the linear P^1, P^3-(diadenosine 5')-1-thiotriphosphate **43** [42] (Scheme 5). The third and fourth of these products corresponded to the *Sp* and *Rp* isomers of 5'-diphosphoryl-adenylyl-(2'→5'-thiophosphoryl)-adenosine (pp5'A2'p_S5'A) **44**. In this case we were not able to detect any formation of the corresponding 3'→5' dinucleotide. Yields and ^{31}P-NMR characteristics [42] are summarized in Tables 1 and 2, respectively. It is noteworthy that in step (v), the formation of the P^1, P^2-(diadenosine 5')-cyclometatriphosphite intermediate **50** (Fig. 4) was evident from the emergence of the ^{31}P multiplet covering the range of -21.4 to -23.0 ppm, which can be attributed to the formation of the P^2(V) and P^3(V) phosphates in **50** (unfortunately, the P^1(III) component was not possible to assign in the downfield region). In the

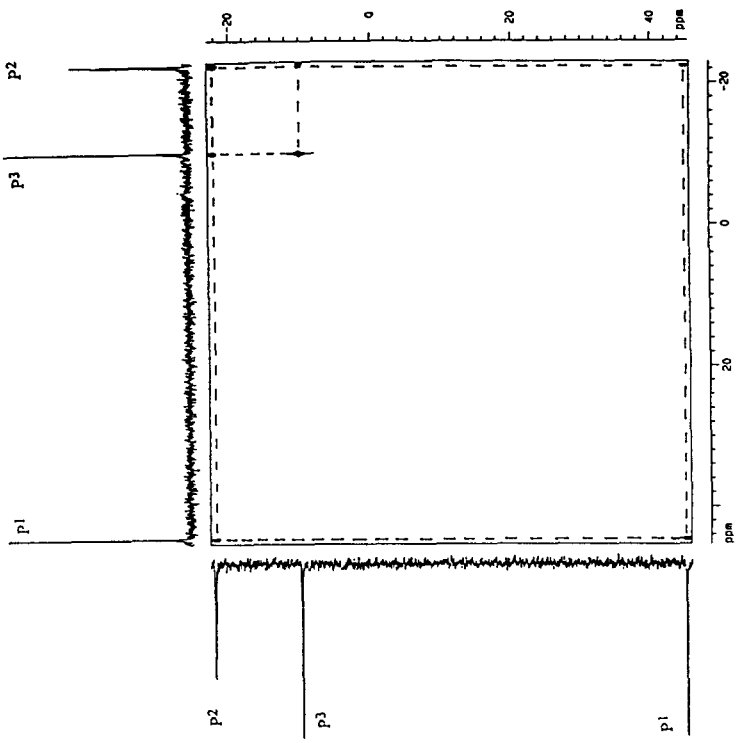


Figure 3. Two dimensional ^{31}P-^{31}P chemical shifts correlation spectrum of ApsppA (43) (*Rp*-isomer) in D_2O at 298K. The phosphate P1 at 44.6 ppm shows a correlation with the phosphate P2 at -22.4 ppm. Phosphate P2 is in turn connected to the phosphate P3 at -10.1 ppm.

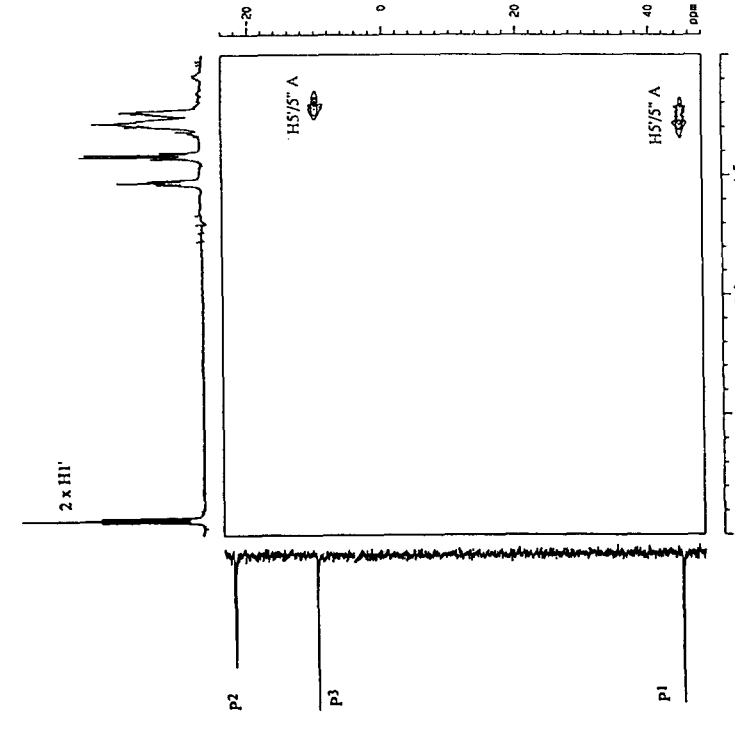


Figure 2. Two dimensional ^{1}H-^{31}P chemical shifts correlation spectrum of ApsppA (43) (*Rp*-isomer) in D_2O at 298K. The phosphate P1 at 44.6 ppm shows a correlation with H5'/5" of adenosine at 4.28 ppm & 4.22 ppm. The phosphate P3 at -10.1 ppm is connected with H5'/5" of adenosine at 4.21 ppm.

Table 1: Experimental conditions showing the effect of the change in the solvent system on the ratio of products formed in Scheme 5.

Expt. No	Activated[a] Nucleotide	Attacking[b] Nucleophile	Solvent system A	Solvent system B	Ratio A:B	Product[c] (% Yield)		
1	**34**	ADP	MeCN	DMF	2:1	**43** (10 %)	**44** (11 %)	
2	**34**	ATP	MeCN	DMF	2:1	**45** (19 %)	**46** (16 %)	
3	**34**	ATP	CH_2Cl_2	DMF	1:1	**45** (4 %)	**46** (24 %)	
4	**34**	ADP	CH_2Cl_2	DMF	2:1	**47** (3 %)	**48** (13 %)	**49** (5 %)
5	**34**	$P_2O_7^{4-}$	MeCN	DMF	1:1	**40** (6 %)	**41** (33 %)	**42** (8 %)
6	**34**	$P_2O_7^{4-}$	CH_2Cl_2	DMF	1:1	**40** (7 %)	**41** (14 %)	**42** (23 %)
7	**34**	$P_2O_7^{4-}$	DMF	DMF	1:1	**40** (7 %)	**41** (30 %)	**42** (7 %)

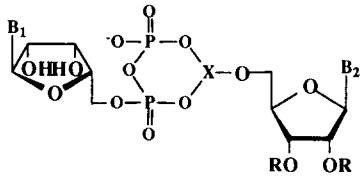
[a]**34** and **35** were obtained by treating dried N-6-benzoyl-2',3'-di-O-acetyladenosine and 2-N-(4,4'-dimethoxytrityl)-N-7-methyl-2',3'-O-methoxymethyleneguanosine inner salt respectively with **23** activated by MAC in solvent A. [b]Tri-n-butylammonium salts of $P_2O_7^{4-}$, ADP & ATP were prepared, coevaporated with solvent B and dissolved in solvent B. [c]The yields given are the combined yields of S_P and the R_P isomers of each product.

sulfurisation reaction of **50** → **51** [step (vii)], the appearance of the triplet at +43.8 ppm furthermore indicated that the phosphorothioate P^1(V) is coupled to the two vicinal phosphates P^2 & P^3 as would be expected from a 1-thiometatriphosphate skeleton in **51**. More unequivocal evidence for the occurrence of cyclometatriphosphite and the corresponding thiometatriphosphate intermediate in the reaction of **34** with a binucleophile (ADP or ATP) was obtained in the corresponding reaction with pyrophosphate (see below). The presence of the 1-thiotetraphosphate chain in the S_P and R_P isomers of Ap_SppA **43** was substantiated by the use of 2D ^1H - ^{31}P- and ^{31}P - ^{31}P correlation spectroscopy (Figs. 2 & 3).

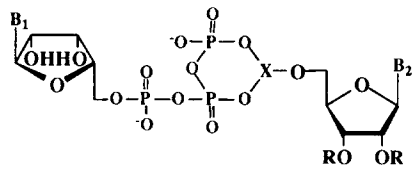
*(b) Reaction of putative intermediate **34** with ATP (Scheme 5) :* ^{31}P-NMR of the reaction mixture containing **34** and ATP [step (vi)] showed the appearance of downfield absorbances at +153.0 ppm (s), +138.0 ppm (s) and +130.0 ppm (m) in a 2 : 3 : 1 ratio. Amongst the upfield absorbances, we observed a new singlet at -23.7 ppm, which is similar to the one formed in the reaction of **34** with ADP. The sulfurisation [step (vii)] caused all the downfield absorbances to shift to +85.9 ppm (s), +83.4 ppm (s), +57.7 ppm (s), +56.7 ppm (s) and a multiplet appeared at +44.0 ppm in 2 : 2 : 1 : 1 : 2 ratio. The hydrolysis [step (viii)] led to the disappearance of the +85.9 and +83.4 ppm absorbances, which obviously shifted to a set of closely grouped singlets at +57.0 ppm. The +44.0 ppm absorbance remained practically intact. The ratio between these two latter groups of absorbances was 1.0 : 1.2. Ammonolysis [step (ix)] and then purification of the residue on DEAE-Sephadex A-25 gave three pure products: the first two components to elute corresponded to the S_P (fast) and R_P (slow) isomers of 5'-triphosphoryl-adeninyl-(2'→5'-thiophosphoryl)-adenosine (ppp5'A2'p_S5'A) **46**[43], whereas the third product was identified by ^1H-NMR, ^{31}P-NMR and mass spectroscopy as the desired R_P / S_P mixture of the linear P^1, P^4-(diadenosine 5')-1-thiotetraphosphate **45** (Scheme 5). All yields are shown in Table 1. We observed that when reactions [steps (i), (iii), (vi)-(ix)] were performed in CH_2Cl_2 instead of MeCN, the yield of **53** increased at the expense of **45** (Table 1). The only P^1, P^n-(diadenosine 5')-1-thiooligophosphate that

was isolated from the reaction sequence [steps (i), (iii), (vi)-(ix)] was the linear Ap_SpppA **45**. This result shows that the attack by ATP on **34** in reaction step (iv) took place exclusively via the P^2, P^3 phosphates of ATP. It also shows that the nucleophilic attack by water occured exclusively at the branched phosphate P^2 of the 1-thiocyclotriphosphate **55** (Fig. 4).

The presence of the 1-thiotetraphosphate chain in the *Sp* and *Rp* isomers of Ap_SpppA **45** was proved by the use of 2D ^1H - ^{31}P- and ^{31}P - ^{31}P correlation spectroscopy (Figs. 5 & 6). The presence of the triphosphate chain in the *Sp* and *Rp* isomers of ppp5'A2'p_S5'A **46** was shown by ^{31}P - ^{31}P correlation spectroscopy (Fig. 8) and the connectivity of the sugar residue to the P^1 phosphate and the P_S phosphorothioate was shown by ^1H - ^{31}P correlation spectroscopy (Fig. 7).



50 : $B_1 = B_2 = A^{Bz}$; R = Ac ; X = P
51 : $B_1 = B_2 = A^{Bz}$; R = Ac ; X = (P=S)
52 : $B_1 = A^{Bz}$; $B_2 = m^7G^{Dmtr}$;
R = 2',3'-O-mM ; X = P
53 : $B_1 = A^{Bz}$; $B_2 = m^7G^{Dmtr}$;
R = 2',3'-O-mM ; X = (P=S)



54 : $B_1 = B_2 = A^{Bz}$; R = Ac ; X = P
55 : $B_1 = B_2 = A^{Bz}$; R = Ac ; X = (P=S)

Figure 4

In our synthesis of the 5'-terminal cap structure m^7Gp_SppA **47**[44], we used a procedure similar to the one used for Ap_SppA, but with some modification. The initial steps (i) and (iv) in Scheme 5 for the generation of the active intermediate **35** were carried out in dry CH_2Cl_2, since the reaction of 2-*N*-(4,4'-dimethoxytrityl)-*N*-7-methyl-2',3'-*O*-methoxymethylene guanosine inner salt (see experimental) with **26A** in dry MeCN was unsuccessful to generate intermediate **35**. The steps (i) and (iv) in CH_2Cl_2 also required longer time to reach completion [2 h to generate **26A** ($\delta^{31}P$ = +125.8 ppm) and 120 min to generate **35** ($\delta^{31}P$ = +123.7 & +122.0 ppm)]. The subsequent three reactions on **35** [steps (v), (vii) and (viii)], *i.e.* reaction with ADP, sulfur and then water showed a very similar absorbance pattern in the ^{31}P-NMR spectrum as in the case of Ap_SppA. The ^{31}P-NMR of the final deprotected mixture showed that the linear m^7Gp_SppA **47**, (Scheme 5), corresponding to the absorbance at +43.8 ppm, had been formed in quite small amount, compared to 2'→5' and 3'→5' dinucleotide adducts **48** and **49** ([**47** and (**48**+**49**), 1 : 5], Scheme 5, Table 2). DEAE-Sephadex A-25 followed by RP-HPLC chromatography separated the *Sp*-isomer from the *Rp*-isomer of **48**. Yields and ^{31}P-NMR characteristics[42] are summarized in Table 1 & 2.

The presence of the 1-thiotriphosphate chain in the *Sp* and *Rp* isomers of m^7Gp_SppA (**47**) was conveniently proved by the use of 2D ^1H - ^{31}P- and ^{31}P - ^{31}P correlation spectroscopy (Figs. 9 & 10). The connection of the sugar residue to the P^1 phosphate and the P_S phosphorothioate in the *Sp* and *Rp* isomers of pp5'A2'p_S5'm^7G (**48**) was shown by ^1H - ^{31}P correlation spectroscopy (Fig. 11)

In the above syntheses of the target compounds **43**, **45** and **47**, a fast eluting material (15 - 20 %) was obtained from the DEAE-sephadex A-25 separation, which was identified as 5'-*H*-phosphonate of adenosine and m^7guanosine, which were characterized as usual by ^1H and ^{31}P-NMR spectroscopy.

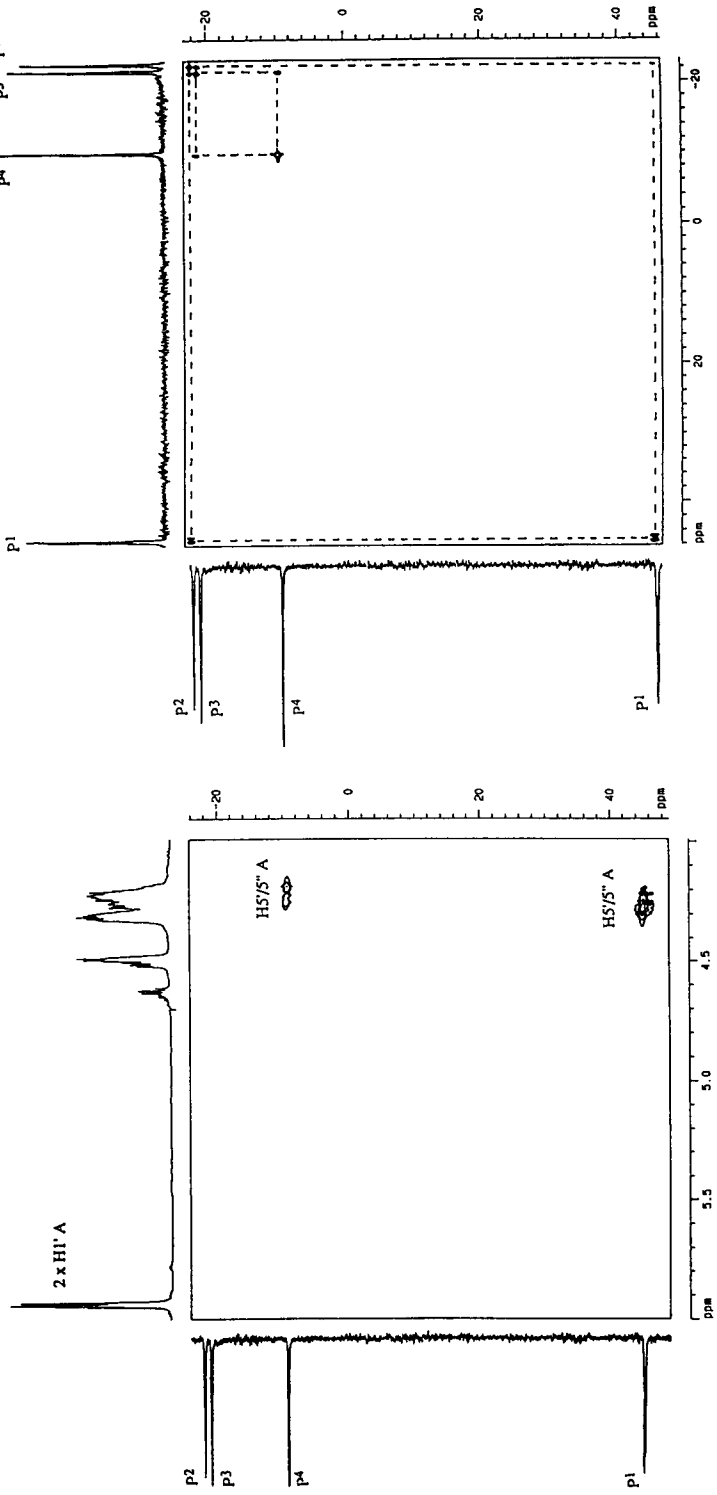


Figure 5. Two dimensional ^{1}H-^{31}P chemical shifts correlation spectrum of Ap_SpppA (45) (*Sp*/*Rp*-isomers) in D_2O at 298K. The phosphate P^1 at 45.2 ppm (*Sp*) & 45.0 ppm (*Rp*) shows a correlation with H5'/5" of adenosine at 4.28 ppm. The phosphate P^4 at -9.3 ppm (*Sp*) & -9.4 ppm (*Rp*) are connected with H5'/5" of adenosine at 4.22 ppm.

Figure 6. Two dimensional ^{31}P-^{31}P chemical shifts correlation spectrum (at 202.45 MHz ^{31}P) of Ap_SpppA (45) (*Sp*/*Rp*-isomers) in D_2O at 298K. The phosphate P^1 at 45.2 ppm (*Sp*) & 45.0 ppm (*Rp*) shows a correlation with the phosphate P^2 at -22.3 ppm. That phosphate P^2 in turn is connected to the phosphate P^3 at -21.3 ppm. Finally, the phosphate P^3 at -21.3 ppm is correlated with the phosphate P^4 at -9.3 ppm (*Sp*) & -9.4 ppm (*Rp*).

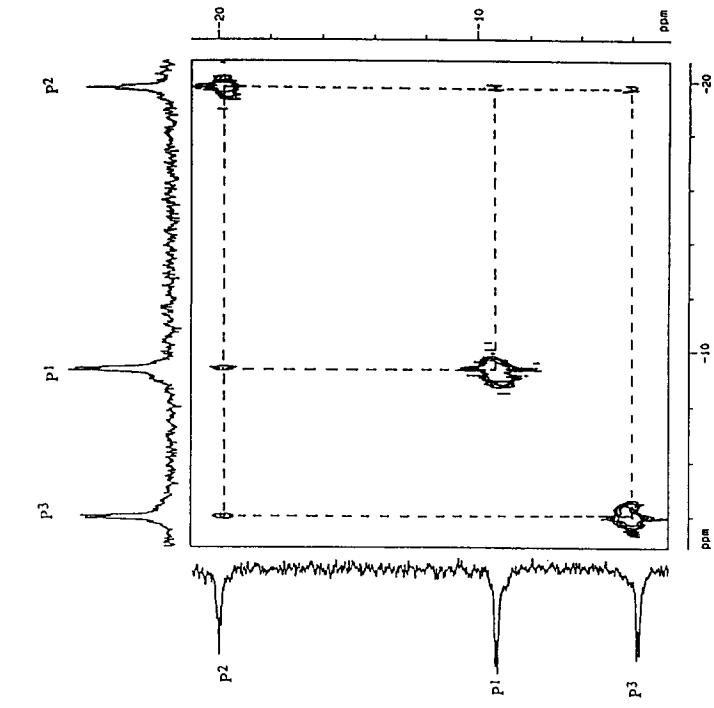


Figure 8. Two dimensional ^{31}P-^{31}P chemical shifts correlation spectrum (at 202.45 MHz 31P) of ppp5'A2'p$_S$5'A (**46**) (*Rp*-isomer) in D_2O at 298K. The 2'→5' linked phosphorothioate P_S at 57.3 ppm (not shown) exhibited no correlation with any other phosphates. The phosphate P^2 at -19.8 ppm is connected to two other phosphates P^1 at -9.3 ppm and P^3 at -4.0 ppm respectively.

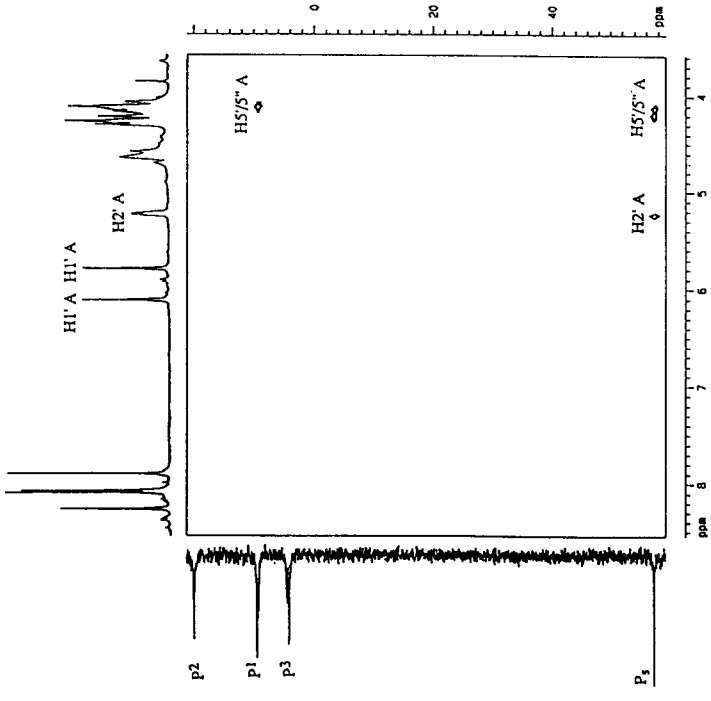


Figure 7. Two dimensional ^{1}H-^{31}P chemical shifts correlation spectrum of ppp5'A2'p$_S$5'A (**46**) (*Rp*-isomer) in D_2O at 298K. The 2'→5' linked phosphorothioate P_S at 57.3 ppm shows correlation with an H2' at 5.39 ppm and with H5'/5'' at 4.14 and 4.01 ppm. The phosphate P^1 at -9.3 ppm shows a correlation with H5'/5'' at 4.19 and 4.00 ppm.

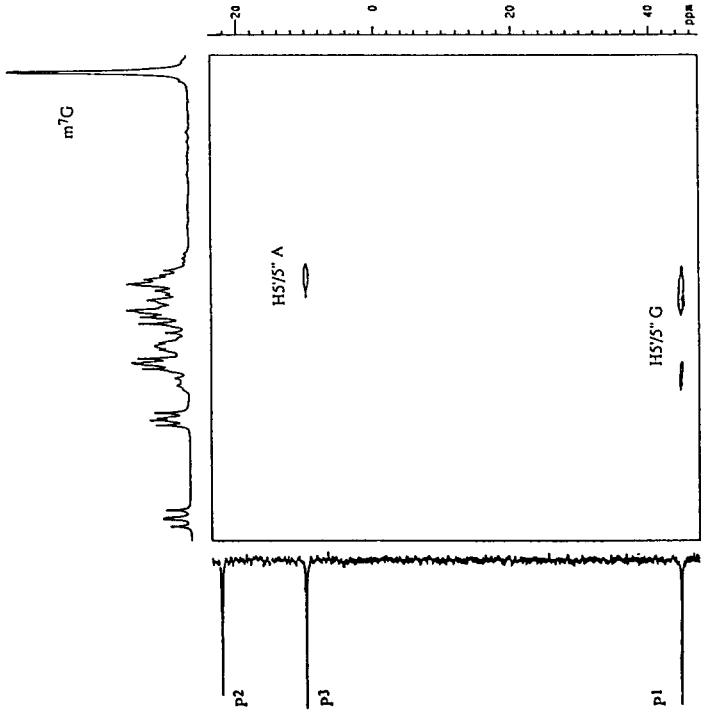


Figure 9. Two dimensional ^1H-^{31}P chemical shifts correlation spectrum of m7GpsppA (47) (R_P-isomer) in D_2O at 298K. The phosphate P1 at 44.5 ppm shows a correlation with H5'/5" of N-7-Me-guanosine (m7G) at 4.25 ppm. The phosphate P3 at -10.1 ppm is connected with H5'/5" of adenosine at 4.25 & 4.37 ppm.

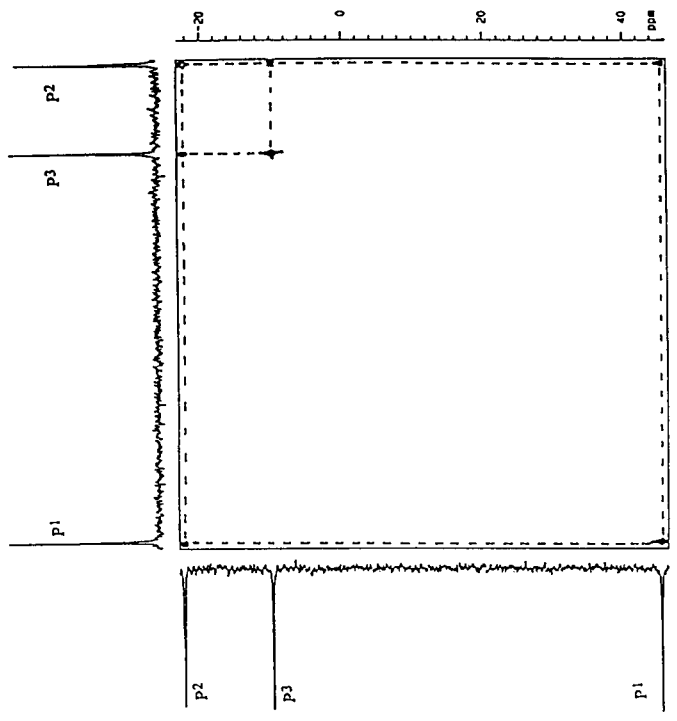


Figure 10. Two dimensional 31P-31P chemical shifts correlation spectrum of m7GpsppA (47) (R_P-isomer) in D_2O at 298K. The phosphate P1 at 44.5 ppm shows a correlation with the phosphate P2 at -22.5 ppm. Phosphate P2 is in turn connected to the phosphate P3 at -10.1 ppm.

Table 2. ^{31}P NMR Spectral data of oligothiophosphates **40** - **49**

		Chemical shifts (ppm)					Coupling constants (Hz)			
		P1	P2	P3	P4	P^5/P_S[a]	$J_{P^1P^2}$	$J_{P^2P^3}$	$J_{P^3P^4}$	$J_{P^4P^5}$
Ap_Sp (**40**)	S_P/R_P	41.8†	-9.65†	-	-	-	26.6	-	-	-
Ap_Spp (**41**)	S_P/R_P	42.9†	-23.0††	-6.8†	-	-	27.9	19.5	-	-
Ap_Spppp (**42**)	S_P	46.5†	-23.3††	-20.6††	-19.8††	-6.6†	24.3	ab	ab	12.7
	R_P	46.0†	-22.8††	-21.3††	-19.3††	-5.3†	26.0	13.0	ab	12.0
Ap_SppA (**43**)	S_P	44.7†	-22.7††	-10.0†	-	-	24.4	20.0	-	-
	R_P	44.6†	-22.4††	-10.1†	-	-	26.0	20.5	-	-
Ap_SpppA (**45**)	S_P	45.2†	-22.3††	-21.3††	-9.3†	-	25.4	16.5	17.2	-
	R_P	45.0†	-22.3††	-21.3††	-9.4†	-	25.6	16.3	17.7	-
m^7Gp_SppA (**47**)	S_P	44.9†	-22.5††	-9.9†	-	-	26.6	20.2	-	-
	R_P	44.5†	-22.5††	-10.1†	-	-	25.8	20.1	-	-
pp5'A2'p_S5'A (**44**)	S_P	-9.2†	-4.5†	-	-	58.6π	22.3	-	-	-
	R_P	-11.2†	-6.7†	-	-	57.5π	20.1	-	-	-
ppp5'A2'p_S5'A (**46**)	S_P	-9.5†	-20.3††	-6.5†	-	58.9π	19.1	16.2	-	-
	R_P	-9.3†	-19.8††	-4.0†	-	57.3π	16.8	10.0	-	-
pp5'A2'p_S5'm^7G (**48**)	S_P	-9.1†	-9.7†	-	-	58.2π	22.0	-	-	-
	R_P	-10.9†	-10.9†	-	-	57.7π	17.0	-	-	-
pp5'A3'p_S5'm^7G (**49**)	S_P	-10.7†	-10.7†	-	-	55.9†	20.2	-	-	-
	R_P	-9.2†	-9.2†	-	-	58.0†	17.6	-	-	-

π denotes a singlet. † denotes a doublet. †† denotes a quartet. ab denotes could not be found. [a] P^5 denotes the terminal phosphate of Ap_Spppp (**42**) while P_S the 2'→5' phosphorothioate diester in case of **44**, **46** & **48** and 3'→5'-phosphorothioate diester in case of **49**.

We have extended this "cyclic approach"[45-49] to also include the reaction of pyrophosphate with our versatile putative intermediate **34** with a goal for the synthesis of adenosine 5'-(1-thiotriphosphate) **41** in a similar manner that Eckstein *et al*[6, 7] achieved with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**13**). While Eckstein isolated 5'-(1-thiotriphosphates) **41** in ~60-70% yield using **13**, we in our reaction of **34** with pyrophosphate, isolated adenosine 5'-(1-thiotriphosphate) **41** (33%) and adenosine 5'-(1-thiopentaphosphate) **42** (23%) in a relatively poorer yield using a procedure that is very similar to the reaction of **34** with ADP or ATP. The presence of **42** indicates that the slight excess of pyrophosphate used in the reaction was able to compete with water during the hydrolysis step and open the 1-thiocyclophosphate ring of **39**, whereas product **40** may have resulted from hydrolysis of **41** and **42**. Fig. 12 shows the presence of five linear phosphate moieties, which was evident through the observed ^{31}P - ^{31}P couplings as would be expected from Ap_Spppp (**42**).

We have subsequently investigated the reaction of **33** with pyrophosphate by ^1H coupled and decoupled ^{31}P-NMR spectroscopy: ^{31}P-NMR spectroscopy of the reaction mixture containing **33** and pyrophosphate showed a doublet at -20.7 ppm and a triplet centered at +104.6 ppm ($J_{P(V)\text{-}O\text{-}P(III)}$ = 43.9 Hz)

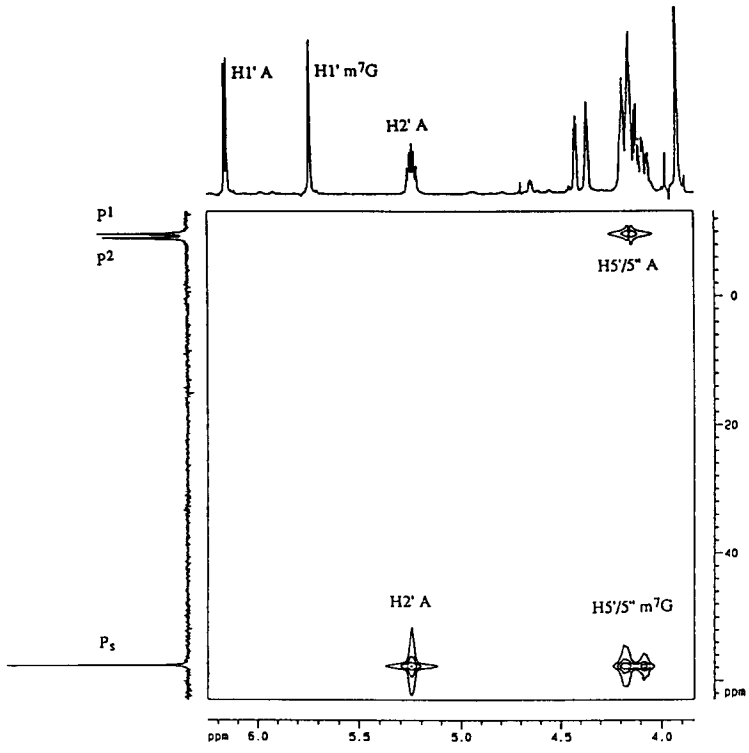


Figure 11. Two dimensional ^{1}H-^{31}P chemical shifts correlation spectrum of pp5'A2'p$_S$5'm^{7}G (48) (*Rp*-isomer) in D_2O at 298K. The 2'→5' linked phosphorothioate P_S at 57.7 ppm shows correlation with an H2' of adenosine at 5.24 ppm and with H5'/5" of N-7-Me-guanosine (m^{7}G) at 4.18 and 4.08 ppm. The phosphate P^1 at -9.7 ppm shows a correlation with H5'/5" of adenosine at 4.12 ppm.

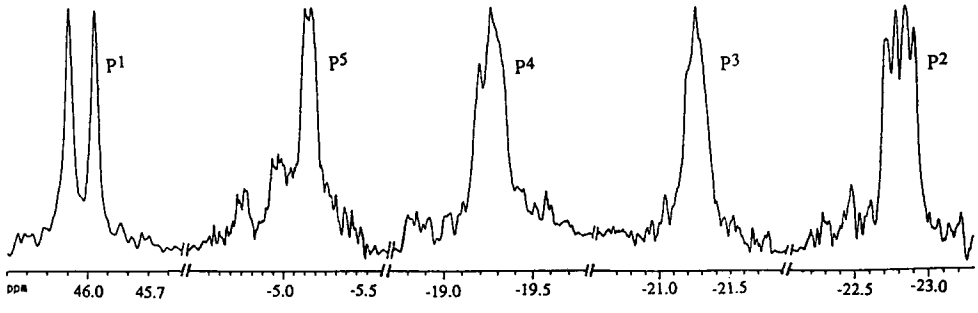


Figure 12. Selected regions of the 1D ^{1}H-decoupled ^{31}P-NMR spectrum (at 202.45 MHz, 4000 scans) of Ap$_S$pppp (42) (*Rp*-isomer) recorded at 298K in 5 mM D_2O solution saturated by $MgCl_2$ (^{31}P resonance of cAMP has been set to 0.0 ppm and used as reference). The phosphate P^1 at 46.0 ppm shows a coupling ($J_{P1,P2} \approx 26$ Hz) with the phosphate P^2 at -22.8 ppm, which is in turn coupled to the phosphate P^3 at -21.3 ppm ($J_{P2,P3} \approx 13$ Hz). The phosphate P^5 at -5.3 ppm is coupled with the phosphate P^4 at -19.3 ppm ($J_{P4,P5} \approx 12$ Hz). The assignment of the P^1, P^2, P^3, P^4 and P^5 phosphorous resonnances was also confirmed by 2D ^{31}P-^{31}P correlation spectrum of 42 (*Rp*-isomer) (see experimentals for nomenclature).

(Fig. 13A) suggesting the formation of the intermediary cyclometatriphosphi(a)te **36** (Scheme 5), which was also found in the reaction of **13** with ethanol and pyrophosphate[6]. In the ^{1}H-coupled spectrum of **36** each line of the triplet at +104.6 ppm was split into a triplet with J_{HCOP} = 8.5 Hz (Fig. 13B), which verified that the trivalent phosphorous was linked to O-CH_3 group. Preparation of corresponding adenosine derivatives **37** (Scheme 5) also gave similar ^{31}P-NMR patterns (Fig. 13C) [triplet at 105.5 ppm ($J_{P(V)\text{-}O\text{-}P(III)}$ = 44.5 Hz, J_{HCOP} = 4.5 Hz) and a doublet at -21.1 ppm). This clearly shows that both our activated reagent **26** and 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**13**) in their alkylphosphite form undergo double-displacement reaction with a binucleophile through the same cyclometatriphosphi(a)te intermediate.

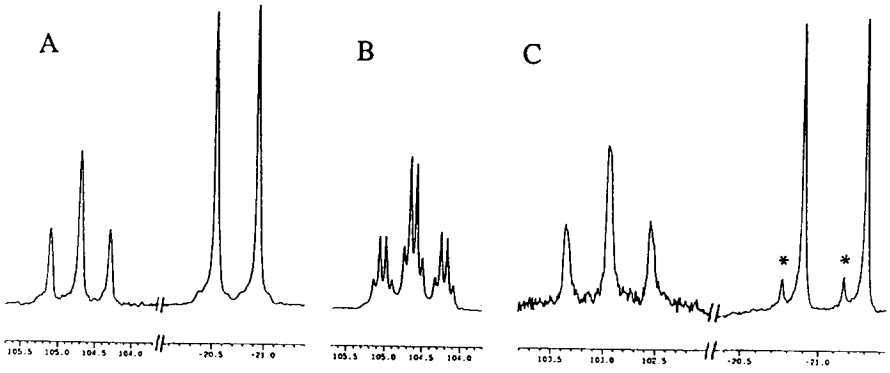


Figure 13. (A) Selected regions of the ^{1}H-decoupled-^{31}P-NMR spectrum of P^1-Methyl P^2,P^3-Dioxocyclo triphosphite **36**: δ_{P1} = +104.6 ppm; δ_{P2} = -20.7 ppm; $J_{P1,P2}$ = 43.9 Hz. (B) Selected region of the ^{1}H-coupled-^{31}P-NMR spectrum of **36**. $J_{P1,H}$ = 8.5 Hz. (C) Selected regions of the ^{31}P-NMR spectrum of **37**. δ_{P1} = +105.5 ppm; δ_{P2} = -21.1 ppm; $J_{P1,P2}$ = 44.5 Hz. * denotes impurity.
The spectra was recorded of reaction mixtures prepared as described in the experimental. Parameters were as follows : Offset, 19230 Hz; sweep width, 20000 Hz; pulse width, 12.0 µs; acquisition time, 1.0 s; line broadening, 1.5 Hz; number of transients, 100 for spectrum 4A & 4B and 500 for spectrum 4C.

CONCLUSION

The reactions of **34** and **35** with ADP and ATP provide one-pot syntheses of Ap_SppA **43**, Ap_SpppA **45** and m^7Gp_SppA **47**, according to the "cyclic approach"[6,7,45-49] which is different from other previous syntheses, which are based upon the "linear approach" [16, 50-53], in which the blockwise linear condensations of mono- or diphosphate blocks take place. This cyclic approach paves the way to the synthesis of many different types of phosphate analogues of nucleoside 5'-oligophosphates and P^1, P^n-(diribonucleoside 5')-oligophosphates, by oxidising the cyclic P(III) intermediate **37**, **50**, **52** or **54** in different oxidation media such as S_8 / pyridine, I_2 / H_2O, I_2 / ROH, I_2 / RNH_2, BH_3-complexes, selenides, etc. Further possiblities of derivatisation can be achieved during the subsequent hydrolytic ring opening of the cyclic P(V) intermediates by replacing water with other nucleophilic solvents and reagents such as Li_2S, H_2S, NH_3, RNH_2, tetrabutylammonium fluoride, etc.

We found that the product distribution of Ap_Spp **41** versus Ap_Spppp **42** was influenced by the choice of the solvent during the initial reaction steps (*i.e.* **23** → **26** → **34**). Shifting from MeCN to CH_2Cl_2 reduces the yield of **41** from 33% to 14%, whereas the yield of **42** changes from 8% to 23% (Table 1, entries 5 and 6). Analogously, the product distribution between Ap_SppppA **45** and ppp5'A2'p_S5'A **46** was influenced upon changing from MeCN (**45**: 19%; **46**: 16%) to CH_2Cl_2 (**45**: 4%; **46**: 24%) (Table 1, entries 2 and 3).

The moderate yields for **45** and **41** (20-33%) and low yields for **43** and **47** (2-10%) was mainly because of the partial decomposition of the intermediates **26** and **34** or **35** during the initial reaction steps, because of the hygroscopic nature of MAC as well as owing to the water of crystallisation of ADP & ATP, which clearly was not possible to remove quantitatively. Furthermore, the use of unprotected ADP and ATP also generated the 2'→5' and 3'→5' dinucleotide adducts **44**, **46**, **48**, & **49**. The poor yield of **47** can be further attributed to the unprotected zwitterionic guanine residue, which can possibly interact with the benzoxazaphosphole **33** in the second step [i.e. step (iv) in Scheme 5], giving rise to additional by-products. These moderate to low yields may to some extent be justifiable by the easy access of our reagent **23** as well as the reactants involved and by the simplicity and flexibility of the above one-pot multicomponent reaction (MCR)[54]. Furthermore, the yields of the oligophosphates can be improved if 2'- and 3'-hydroxyls of ADP or ATP are suitably protected.

In conclusion, we have introduced the present "cyclic approach" for the synthesis of P^1,P^n-(dinucleoside 5')-oligophosphates of general structures r(Np_SppN), r(Np_SpppN), r(m^7Gp_SppN), as well as ribonucleoside 5'-oligophosphates of general structure rNp_Spp and rNp_Spppp, by applying the new phosphitylating agent **23**. This approach does not only have the potential to give the thio analogues, which we have focused on in this work, but also gives the possibility of synthesizing a large variety of other phosphate analogues of P^1, P^n-(diribonucleoside 5')-oligophosphates and ribonucleoside 5'-oligophosphates.

EXPERIMENTAL

^{1}H-NMR spectra were recorded in δ scale with Jeol FX 90 Q, Jeol JNM-GX 270, Bruker AM 360 and Bruker AMX-500 spectrometers at 90, 270, 360 and 500 MHz respectively, using TMS or H_2O (set at 4.7 ppm) as internal standards. ^{31}P-NMR spectra were recorded at 36, 109 and 202 MHz using 85% phosphoric acid and 3', 5'-cAMP (at 202 MHz) as external standard. For all 2D experiments ^{31}P resonance of 3', 5'-cAMP has been set to 0.0 ppm and used as reference. All spectra were recorded at 298K in 10 mM D_2O solution. The ^{31}P-^{31}P corelation spectra were recorded with a sweep of 91 ppm and with ^{1}H decoupling. 8K complex data points were used in F2 dimension and 512 experiments of 48 scans in the F1 dimension. The relaxation delay were 2s. Before Fourier transformation a sinesquare window was applied in both dimensions and the spectra were zero-filled to 4K by 2K real data points. The ^{1}H-^{31}P correlated spectra were recorded with a sweep of 10 ppm and 4K complex data points in the F2 dimension and with 512 experiments of 32 scans in the F1 dimension (sweep of 88 ppm). The relaxation delay were 3s and a delay of 60 ms were used (J_{HP} = 8.3 Hz). The spectra were then zero-filled and a sinesquare window were applied in both dimensions before Fourier transformation, giving a final spectra of 2K by 2K real data point. Jeol DX 303 instrument was used for recording mass spectra, operating at low resolution.

The second order rate constants k were obtained by first following by integration the disappearance of the two doublets around 1.20 and 1.22 ppm of the *N,N*-diisopropylamino group of **23** and the appearence of the doublet at 1.40 ppm of the free *N, N*-diisopropylammonium chloride. At the same time we also observed the chemical shift change taking place for the $MeSO_2N$-group, from around 2.90 ppm of **22**, **23** or **24** to 3.05 ppm of **25A**, **26A** or **27A** (Scheme 1). The mole fractions were plotted against time and a best-fit slope was obtained from which the k value was calculated.

Dry acetonitrile (MeCN) was prepared[55] by first storing it overnight over 3 Å molecular sieves and then distilling it from P_2O_5 under argon. *N,N*-Dimethylformamide (DMF) was sequentially dried[56] over 3 Å molecular sieves after distilling it from P_2O_5 under argon. Dichloromethane was distilled from P_2O_5 under argon. Methanol was distilled[57] from Mg/I_2 onto 3 Å molecular sieves and then allowed to stand for 48 h.

Dry tri-n-butylamine was obtained by distillation from CaH_2 under argon. All solvents were stored over 4 Å molecular sieves in supersealed bottles (Aldrich). Dry diethyl ether was purchased from Merck.

Thin layer chromatography was carried out using pre-coated Merck silica gel F254 TLC in the following CH_2Cl_2-MeOH mixtures: (A) 9 : 1 (v/v), (B) 8 : 2 (v/v). DEAE-Sephadex A-25 from Pharmacia was used for the ion exchange chromatography. An LDC equipment with ConstaMetric Pump model III and Gradient Master was used for analytical HPLC chromatography. A Gilson equipment with Pump Model 303, Manometric Module Model 802C and Dynamic Mixer 811B (23 ml volume) connected to a Dynamax computer program for gradient control was used for semi-preparative RP-HPLC separations. Analytical HPLC and high pressure semi-preparative Spherisorb S50DS2 column chromatography were carried out using gradients of solvent B (50% MeCN in 0.1 M triethylammonium acetate (TEAA)) in solvent A (5% MeCN in 0.1 M TEAA). The *RP* & *SP* diastereomers of the thiooligophosphates were separated with RP-HPLC. In all cases, the faster eluting isomer[6, 42] was designated *SP* and the slower eluting isomer *RP*. All reactions were carried out at RT, unless otherwise specified.

Tri-n-butylammonium salts of ATP, ADP or pyrophosphate were prepared according to a reported procedure[6]. In the preparation of tri-n-butylammonium salts of ATP & ADP, the residue obtained was dissolved in water (10 ml), transfered into small vials and lyophilized to give a white solid. The tri-n-butylammonium salts of ATP & ADP were stored at -20 °C and were prior to use coevaporated with dry DMF (4 x 2 ml). *N*-methylanilinium hydrochloride (MAC) was collected as white crystals by bubbling dry HCl gas through a cooled solution of *N*-methylaniline in dry ethyl ether.

The α-phosporothioate in the linear phosphate chain of **40-42, 43, 45, 47** has been designated as P^1. In the 2'→5' and 3'→5' dinucleotide adducts **44, 46, 48, & 49**, the phosporothioate has been designated as P_S. In these adducts the phosphate next to the nucleoside has been designated as P^1.

2-(*N*,*N*-diisopropylamino)-2, 3-dihydro-3-(methylsulfonyl)-1, 3, 2-benzoxazaphospholes (22), (23) & (24). To a solution of dichloro-(*N*,*N*-diisopropylamino)phosphine (1 eq.) and Et_3N (1 eq.) in dry diethyl ether, one equivalent each of either **19, 20** or **21** in dry diethyl ether was added from a dropping funnel during 15 min. After stirring overnight the amine hydrochloride was filtered off. The filtrate was evaporated in vacuo and the remaining brown residue in each case was crystallised from diethyl ether /hexane mixture. Filteration was repeated if the filterate was found to be turbid. The benzoxazaphospholes **22, 23** and **24** were obtained as white, pale orange and yellow solids respectively in quantitative yield. **22 :** ^{1}H-NMR (360 MHz, $CDCl_3$): 7.50 (d, 1H), 6.97 (m, 3H) aromatic; 3.25-3.38 (m, 2H) 2x-C*H*$(CH_3)_2$; 3.04 (s, 3H) SO_2CH_3; 1.27 (m, 12H) 2x-C*H*$(CH_3)_2$. ^{31}P-NMR (36 MHz, $CDCl_3$) : +131.5 ppm. **23 :** ^{1}H-NMR (360 MHz, $CDCl_3$): 7.45 (d, 1H), 6.97 (dd, 1H), 6.86 (d, 1H) aromatic; 3.27-3.36 (m, 2H) 2x-C*H*$(CH_3)_2$; 2.95 (s, 3H) SO_2CH_3; 1.23 (m, 12H) 2x-C*H*$(CH_3)_2$. ^{31}P-NMR (36 MHz, $CDCl_3$) : +135.0 ppm. **24 :** ^{1}H-NMR (360 MHz, $CDCl_3$): 7.38 (d, 1H), 7.06 (d, 1H) aromatic; 3.27-3.37 (m, 2H) 2x-C*H*$(CH_3)_2$; 3.08 (s, 3H) SO_2CH_3; 1.26 (m, 12H) 2x-C*H*$(CH_3)_2$. ^{31}P-NMR (36 MHz, $CDCl_3$) : +137.0 ppm.

6-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)adenosine 2',3'-diisopropylammonium cyclic phosphorothioate (28). 6-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)adenosine (100 mg, 0.148 mmol) was rendered anhydrous by coevaporation with dry toluene and was then dissolved in dry CH_2Cl_2 (4 ml). To the solution was added successively dry solid DIPAT (16.5 mg, 0.096 mmol) followed by **23** (67.5 mg, 0.192 mmol). The resultant clear solution was stirred for 38 h. The reaction was then quenched by addition of sublimed sulfur (24 mg, 1.48 mmol) suspended in dry pyridine (0.5 ml) and stirred for 30 min. The reaction mixture was filtered and worked up with 5 % aq NH_4HCO_3 solution and CH_2Cl_2 (3 x 5 ml). The crude mixture after coevaporation with toluene was purified by silica gel column chromatography (0 - 6% EtOH in CH_2Cl_2), which afforded pure **28** (100 mg, 0.117 mmol, 79%), R_f: 0.55 (B). ^{1}H-NMR (270 MHz, $CDCl_3$ + CD_3OD): 8.69 (s, 1H) H8, *SP*; 8.67 (s, 1H) H8, *RP*; 8.23 (s, 1H) H2, *SP*; (8.22 (s, 1H) H2, *RP*; 8.10 - 6.73 (m, 18H) arom.; 6.45 (d, $J_{1',2'}$ = 3.5 Hz, 1H) H1', *RP*; 6.40 (d, $J_{1',2'}$ = 3.9 Hz, 1H) H1', *SP*; 5.74 (m, 1H) H2', *RP*; 5.54 (m, 1H) H2', *SP*; 5.28 (m, 1H) H3', *RP* & *SP*; 4.74 (m, 1H) H4', *RP*; 4.54 (m, 1H) H4', *SP*; 3.77, 3.76 (2xs, 6H) 2 x OCH_3; 3.39 (m, 4H) H5', 5" & CH of (iso-Pr)$_2NH^+$; 1.39 (d, J = 6.5 Hz, 12H) CH_3 of (iso-Pr)$_2NH^+$. ^{31}P-NMR (36 MHz, $CDCl_3$ + CD_3OD): +74.6 ppm (*SP*), +73.1 ppm (*RP*).

4-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(2-chloroethoxyethyl)cytidine 3'-diisopropylammonium *O*-(4-chloro-2-methylsulfonamidophenyl)phosphorothioate (29). 4-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(2-chloroethoxyethyl)cytidine[58] (High + low R_f-isomers) (100 mg, 0.132 mmol) was rendered anhydrous by coevaporation with dry toluene and was then dissolved in dry CH_2Cl_2 (3.5 ml). To the solution was added successively dry solid DIPAT (23 mg, 0.132 mmol) followed by **23** (93 mg, 0.265 mmol). The resultant clear solution was stirred for 7 days. The reaction was then quenched by addition of sublimed sulfur (42 mg, 2.65 mmol) suspended in dry pyridine (0.8 ml) and stirred for 30 min. The reaction mixture was filtered and worked up with 5% aq. NH_4HCO_3 solution and CH_2Cl_2 (3 x 5 ml). The crude mixture after coevaporation with toluene was purified by silica gel column chromatography (2 - 6% EtOH in CH_2Cl_2),

which afforded pure **29** (120 mg, 0.107 mmol, 81%) consisting of four diastereoisomers in approximately 1 : 0.6 : 0.9 : 1.05 ratio, R_f: 0.57 (B). ^{1}H-NMR (270 MHz, $CDCl_3$ + CD_3OD): 8.58 (m, 1H) H6; 8.01-6.82 (m, 22H) arom. & H5; 5.96 (m, 1H) H1'; 5.20 (m, 1H) CH of 1-(2-chloroethoxy)ethyl; 5.00 (m, 1H) H3'; 4.59 (m, 1H) H2'; 4.40 (m, 1H) H4'; 4.07-3.84 (m, 2H) H5', 5"; 3.81 (s, 6H) -OCH_3 of DMTr; 3.73-3.52 (m, 4H) CH_2CH_2 of 1-(2-chloroethoxy)ethyl; 3.35 (m, 2H) 2 x CH of (iso-Pr)$_2NH^+$; 3.02, 2.98, 2.89 (3 x s, 3H) CH_3SO_2-; 1.45 (m, 3H) CH_3 of 1-(2-chloroethoxy)ethyl; 1.33 (d, J = 6.6 Hz, 12H) CH_3 of (iso-Pr)$_2NH^+$. ^{31}P-NMR (36 MHz, $CDCl_3$ + CD_3OD): +57.4, +57.0, +55.5, +55.0 ppm.

Bis[6-*N*, 3'-*O* -bis(4-methoxytrityl)-2'-deoxyadenosine-5']-[5'-*O*-(4-methoxytrityl)thymidine-3']-mono phosphate (31). Benzoxazaphosphole **23** (50 mg, 0.143 mmol) was dissolved in dry MeCN (0.6 ml) under argon and activated by addition of freshly sublimed MAC (41 mg, 0.286 mmol). After 40 min, a solution of 5'-*O*-(4-methoxytrityl)thymidine (66 mg, 0.129 mmol) in dry MeCN (0.15 ml) was added rapidly with a syringe through a rubber septum and the resultant solution was stirred for 25 min. Then a solution of dry 6-*N*, 3'-*O*-bis(4-methoxytrityl)-2'-deoxyadenosine (66 mg, 0.129 mmol) in dry MeCN (0.2 ml) was added in the same way and the resultant solution was stirred for 3 h. During this time two portions of freshly sublimed MAC (20 mg, 0.143 mmol) were added at 1 h intervals. The reaction was then quenched by addition of a 1 M solution of iodine in tetrahydrofuran-pyridine-water (7:2:1 v/v/v) (0.157 ml, 0.157 mmol) and stirred for 10 min. The reaction mixture was poured into an aq. NH_4HCO_3 / $Na_2S_2O_3$ solution and extracted with CH_2Cl_2 (3 x 5 ml). The crude mixture was purified by silica gel column chromatography (0 - 2% EtOH in CH_2Cl_2) to give **31** (50 mg, 0.023 mmol 16 %). R_f: 0.78 (A). ^{1}H-NMR (270 MHz, $CDCl_3$): 7.98, 7.97(2 x s, 1H) H8; 7.83, 7.79 (2 x s, 1H) H2; 7.48-6.71 (m, 71H) arom. & TH6; 6.42-6.29 (m, 3H) TH1' & 2 x AH1'; 5.30 (t, 1H) TH3'; 4.34 (m, 2H) 2 x AH3'; 4.02-3.86 (m, 3H) TH4' & 2 x AH4'; 3.80-3.58 (m, 19H) 5 x OCH_3 of MMTr & 2 x AH5', 5"; 3.30 (d,d, $J_{5',5"}$ = 11.3 Hz, $J_{4',5'}$ = 2.7 Hz, 1H) TH5'; 3.18 (d,d, $J_{4',5"}$ = 2.6 Hz, 1H) TH5"; 2.45-1.88 (m, 6H) TH2',2" & 2 x AH2',2"; 1.33 (s, 3H) T*Me*-5; ^{31}P-NMR (36 MHz, $CDCl_3$): -2.0 ppm.

Bis(2'-deoxyadenosine 5')- thymidine 3'-monophosphate (32). 31 (50 mg, 0.023 mmol) was dissolved in 80% aq. AcOH (3 ml) and stirred for 4 h. The solvent was evaporated and the residue was partitioned between diethyl ether and water. The aqueous phase was evaporated, the residue taken up in EtOH and subjected to preparative thin layer chromatography, using MeCN - water (4 : 1, v/v) as eluent to give **32** (11 mg, 0.014 mmol, 60%). R_f: 0.03 (B). ^{1}H-NMR (270 MHz, $CDCl_3$ + CD_3OD): 8.25, 8.247 (s, 1H) H8; 8.16, 8.14 (s, 1H) H2; 7.63 (d, J = 1.2 Hz, 1H) TH6; 6.41 (t, $J_{1',2'}$ & $J_{1',2"}$ = 6.4 Hz, 2H) 2 x AH1'; 6.24 (d,d, $J_{1',2'}$ = 5.9 Hz, $J_{1',2"}$ = 6.0 Hz, 1H) TH1'; 5.00 (t, 1H) TH3'; 4.60 (m, 2H) 2 x AH3'; 4.38-4.00 (m, 5H) 2 x AH5', 5" & TH4'; 2.81 (m, 2H) 2 x AH2'; 2.60-2.41 (m, 3H) AH2" & TH2'; 2.20 (m, 1H) TH2"; 1.89 (d, J = 1.2 Hz, 3H) TCH_3-5; ^{31}P-NMR (36 MHz, $CDCl_3$ + CD_3OD): -2.3 ppm.

Methyl 1-thiotriphosphate (38) : The aroxazaphosphole **23** (50 mg, 0.14 mmol) was dissolved in dry $CDCl_3$ (200 µl) and treated with a dry 1M solution of MAC in $CDCl_3$ (314 µl, 0.31 mmol) under argon. The reaction was monitered by ^{31}P-NMR and after 40 min, the protonated *N*-methylanilino derivative **26A** was formed. ^{1}H-NMR (270 MHz, $CDCl_3$): 7.5-6.9 (m, 8H) aromatic; 3.1 (s, 3H) SO_2CH_3; 2.8 (d, 3H, J_{HCNP} = 4.6 Hz) -NCH_3Ph. ^{31}P-NMR (36 MHz, $CDCl_3$): +126.18 ppm. Then dry methanol (5.8 µl, 0.14 mmol) was added to the reaction mixture and after 2 min, **33**[12] was formed. ^{1}H-NMR (270 MHz, $CDCl_3$): 7.4 (d, 1H) arom; 7.0 (m, 2H) arom; 3.6 (d, 3H, J_{HCOP} = 10.4 Hz) -OCH_3; 3.0 (s, 3H) SO_2CH_3. ^{31}P-NMR (36 MHz, $CDCl_3$): +123.01 ppm. A dry 0.5 M solution of bis(tri-n-butylammonium) pyrophosphate in DMF (570 µl, 0.29 mmol) and dry n-tributylamine (140 µl, 0.6 mmol) were added to a 1ml RB flask under argon. To this solution, the solution containing **33** was added via a syringe. After 10 min the solution was transfered to a NMR tube flushed with argon, and the ^{31}P-NMR spectrum showed the formation of P^1-Methyl P^2,P^3-Dioxocyclotriphosphite **36**. ^{31}P-NMR (109 MHz, $CDCl_3$/DMF) : δ_A = +104.6 (tdd, J_{AB} = 43.9 Hz, J_{AH} = 8.5 Hz, 1P); δ_B = -20.7 (d, 2P). Then sublimed sulphur (11 mg, 0.7 mmol) was added to the reaction mixture, which resulted in the formation of Methyl 1-thiocyclotriphosphate **38** [^{31}P-NMR (109 MHz, $CDCl_3$/DMF) : δ_A = +44.6 (td, J_{AB} = 34.7 Hz, J_{AH} = 14.7 Hz, 1P); δ_B = -24.4 (d, 2P)].

General Procedure for synthesis of Oligophosphates 40-46 : The benzoxazaphosphole **23** (50 mg, 0.14 mmol) in dry CH_3CN (200 µl) was reacted with a dry 1M solution of MAC in MeCN (314 µl, 0.31 mmol). 6-*N*-benzoyl-2', 3'-di-*O*-acetyladenosine (61 mg, 0.14 mmol) was coevaporated twice with dry CH_3CN, and dissolved in dry CH_3CN (100 µl) and added to the protonated N-methylanilino derivative **26A** under argon. The reaction was monitored by ^{31}P-NMR and showed after 30 min the formation of **34** [^{31}P-NMR (36 MHz): +121.1 and 122.8 ppm]. The reaction mixture was added slowly to a dry solution of tri-n-butylammonium salt of ADP (0.21 mmol), tri-n-butylammonium salt of ATP (0.21 mmol) or bis(tri-n-butylammonium)

pyrophosphate (0.29 mmol) and dry tri-n-butylamine (142 µl, 0.6 mmol) in DMF (300 µl) over 1 h (for pyrophosphate the addition was over 20 min). The reaction mixture was stirred under argon for 10 min. Then sublimed sulfur (11 mg, 0.7 mmol) was added and the mixture was stirred for 20 min. Subsequently water (5 ml) was added and after stirring for 45 min the reaction mixture was evaporated to dryness. The residue was then treated with aqueous ammonia (35 ml) for 6 h. The solution was concentrated and applied to a DEAE-Sephadex A-25 column (2 x 25 cm, HCO_3^- form). The procedure of further purification has been described below for each case separately.

Adenosine-5'-*O*-(1-thiodiphosphate) (40), Adenosine 5'-*O*-(1-thiotriphosphate) (41) & Adenosine 5'-*O*-(1-thiopentaphosphate) (42) : The DEAE-Sephadex A-25 column separation was carried out using a linear gradient 0.001 M - 0.25 M - 0.5 M of aq. NH_4HCO_3 solution (500 ml / 1000 ml / 500 ml respectively; pH 7.5). Three peaks were collected between 0.4 M and 0.5 M which were the products **40, 41 & 42** (yields : Table 1). When **26A** and **34** were generated in dry CH_2Cl_2 and in dry DMF, following the same general procedure, **40, 41 & 42** were obtained in different yields (see Table 1). **40** (*Sp*/*Rp*) : ^{1}H-NMR (270 MHz, D_2O): 8.54, 8.51 (2 x s, 1H) H8; 8.14 (s, 1H) H2; 6.06 (d, $J_{1',2'}$ = 6.3 Hz, 1H) H1'; 4.83 (m, 1H) H2'; 4.66 (m, 1H) H3'; 4.25 (m, 3H) H4', H5', H5". **41** (*Sp*/*Rp*) : ^{1}H-NMR (270 MHz, D_2O) : 8.52, 8.46 (2 x s, 1H) H8; 8.06 (s, 1H) H2; 6.02 (d, $J_{1',2'}$ = 5.1 Hz, 1H) H1'; 4.80 (m, 1H) H2'; 4.54 (m, 1H) H3'; 4.37 (m, 1H) H4'; 4.24 (m, 2H) H5', H5". MS (FAB$^-$): calc for (M-Na$^+$) 603.9 found 603.7 **42** (*Sp*/*Rp*) : ^{1}H-NMR (270 MHz, D_2O) : 8.57, 8.49 (2 x s, 1H) H8; 8.11(s, 1H) H2; 6.05 (d, $J_{1',2'}$ = 4.5 Hz, 1H) H1'; 4.80 (m, 1H) H2'; 4.54 (m, 1H) H3'; 4.24 (m, 3H) H4', H5', H5" (for ^{31}P-NMR see Table 2). MS (FAB$^-$): calc for (M-Na$^+$) 830.8 found 830.7

P^1, P^3-(diadenosine 5')-1-thiotriphosphate (43) & 5'-diphosphoryl-adeninyl-(2'→5'-thiophosphoryl)-adenosine (44) : The DEAE-Sephadex A-25 column separation was carried out using the gradient 0.001 M - 0.4 M - 0.4 M - 0.5 M of aq. NH_4HCO_3 solution (500 ml / 1000 ml / 1000 ml / 500 ml respectively; pH 7.5). Product **44** (*Sp*-isomer) eluted as a symmetrical peak between 0.41 M and 0.42 M (Fraction A). A second peak with a shoulder was collected between 0.43 M and 0.46 M (Fraction B), which was purified by semi-preparative RP-HPLC column chromatography by dissolving batches of 5-7 mg of lyophilized material in 5% MeCN in 0.1M TEAA at pH 7.0 (solvent A) (2 ml) in Eppendorf tubes. The solutions were centrifuged and purified on a semi-preparative Spherisorb S5ODS2 column (8 x 250 mm) pre-equilibrated in solvent A.Fraction B was purified with the following gradient (1 ml / min; mixer volume 23 ml): solvent A (20 min), then a 20 min increase to 5% solvent B. This gradient run gave, (1) pure *Sp*-isomer of **43** (R_t = 37.3 min), (2) pure *Rp*-isomer of **43** (R_t = 59.7 min) and (3) pure *Rp*-isomer of **44** (R_t = 55.5 min). These purified materials were collected, evaporated and then lyophilized several times (~9 x 1 ml) until the TEAA salt was removed (monitored by ^{1}H-NMR). Then they were converted to their Na$^+$ salts through Dowex Na$^+$-form. **43** (*Sp*, Na$^+$-salt) : ^{1}H-NMR (500 MHz, D_2O): 8.38 (s, 1H) H8; 8.20 (s, 1H) H8; 8.01 (s, 2H) 2x H2; 5.92 (d, $J_{1',2'}$ = 4.8 Hz, 1H) H1'; 5.90 (d, $J_{1',2'}$ = 4.7 Hz, 1H) H1';4.76 (m, 2H) 2x H2'; 4.60 (m, 1H) H3'; 4.47 (m, 1H) H3'; 4.31-4.18 (m, 6H) 2x (H4',H5' & H5"). **43** (*Rp*, Na$^+$-salt) : ^{1}H-NMR (500 MHz, D_2O): 8.38 (s, 1H) H8; 8.20 (s, 1H) H8; 8.01 (s, 2H) 2x H2; 5.94 (dd, $J_{1',2'}$ = 4.7 Hz, 1H) H1'; 5.92 (d, $J_{1',2'}$ = 4.7 Hz, 1H) H1';4.53 (2 t, $J_{2',3'}$ = 4.7 Hz, 2H) 2x H2'; 4.42 (q, 1H) 2 x H3'; 4.31-4.22 (m, 6H) 2x (H4',H5' & H5"). **44** (*Sp*, Na$^+$-salt): ^{1}H-NMR (500 MHz, D_2O): 8.35 (s, 1H) H8; 8.18 (s, 1H) H8; 8.11 (s, 1H) H2; 7.97 (s, 1H) H2; 6.14 (d, $J_{1',2'}$ = 4.2 Hz, 1H) H1'; 5.82 (d, $J_{1',2'}$ = 4.2 Hz, 1H) H1'; 5.44 (ddd, $J_{2',1'}$ = 4.2 Hz, $J_{2',3'}$ = 5.6 Hz, $J_{2',P}$= 10.2 Hz, 1H) H2'; 4.75 (m, 1H) H3'; 4.38 (dd, 1H) H2'; 4.36-4.08 (m, 8H) H3' & 2x (H4', H5' & H5"). **44** (*Rp*, Na$^+$-salt): ^{1}H-NMR (500 MHz, D_2O): 8.28 (s, 1H) H8; 8.10 (s, 1H) H8; 8.08 (s, 1H) H2; 7.92 (s, 1H) H2; 6.12 (d, $J_{1',2'}$ = 4.1 Hz, 1H) H1'; 5.80 (d, $J_{1',2'}$ = 4.1 Hz, 1H) H1'; 5.24 (ddd, $J_{2',1'}$ = 4.1 Hz, $J_{2',3'}$ = 5.2 Hz, $J_{2',P}$ = 9.1 Hz, 1H) H2'; 4.70 (m, 1H) H3'; 4.31 (dd, 1H) H2'; 4.34-4.04 (m, 8H) H3' & 2x (H4', H5' & H5").

P^1, P^4-(diadenosine 5')-1-thiotetraphosphate (45) & 5'-triphosphoryl-adeninyl-(2'→5'-thiophosphoryl)-adenosine (46) : The DEAE-Sephadex A-25 column separation was carried out using the gradient 0.001 M - 0.4 M - 0.5 M of aq. NH_4HCO_3 solution (500 ml / 1000 ml / 500 ml respectively; pH 7.5). Three peaks were collected between 0.45 M and 0.5 M, which corresponded to **45** (*Sp*/*Rp*), **46** (*Sp*) & **46** (*Rp*). **45** (*Sp*/*Rp*, Na$^+$-salt): ^{1}H-NMR (500 MHz, D_2O): 8.44 (44%) and 8.34 (56%) (s, 1H) H8:*Rp* & *Sp* ; 8.27 (s, 1H) H8; 8.00 (s, 2H) 2x H2; 5.93 (t of d, $J_{1',2'}$ = 5.6 Hz, $J_{1',2'}$ = 5.5 Hz, $J_{1',2'}$ = 5.5 Hz, 2H) H1' & H1': *Rp* & *Sp* ; 4.66-4.60 (m, 2H) 2x H2'; 4.53-4.46 (m, 2H) 2 x H3'; 4.34-4.16 (m, 6H) 2x (H4',H5' & H5"). MS (FAB$^-$): calc for (M-Na$^+$) 917.0 found 916.9 **46** (*Sp*, Na$^+$-salt) : ^{1}H-NMR (500 MHz, D_2O): 8.17 (s, 1H) H8; 8.08 (s, 1H) H8; 7.99 (s, 1H) H2; 7.78 (s, 1H) H2; 6.04 (d, $J_{1',2'}$ = 3.8 Hz, 1H) H1'; 5.71 (d, $J_{1',2'}$ = 3.0 Hz, 1H) H1'; 5.39 (ddd, $J_{2',1'}$ = 3.8 Hz, $J_{2',3'}$ = 5.2 Hz, $J_{2',P}$ = 9.1 Hz, 1H) H2'; 4.2 (m, 3H) H2', 2x H3'; 4.15-3.95 (m, 6H) 2x (H4', H5' & H5"). MS (FAB$^-$): calc for (M-Na$^+$) 900.0 found 900.1 **46** (*Rp*, Na$^+$-salt) : ^{1}H-NMR (500 MHz, D_2O): 8.20 (s, 1H) H8; 8.03 (s, 1H) H8; 7.99 (s, 1H) H2; 7.84 (s, 1H) H2; 6.06 (d, $J_{1',2'}$ = 4.2 Hz, 1H) H1'; 5.73 (d, $J_{1',2'}$ = 3.9 Hz, 1H) H1'; 5.20 (ddd, $J_{2',1'}$ = 4.2 Hz, $J_{2',3'}$ = 5.4 Hz, $J_{2',P}$ = 9.3 Hz, 1H) H2'; 4.25-3.98 (m, 9H) H2', 2x (H3', H4', H5' & H5").

2-*N*-(4,4'-dimethoxytrityl)-*N*-7-methyl-2',3'-*O*-methoxymethyleneguanosine Inner Salt . Dry 2-*N*-(4,4'-dimethoxytrityl)-2',3'-di-*O*-methoxymethyleneguanosine[59] (626 mg, 1 mmol) was dissolved in dry DMF (14 ml) and then solid $Na_2HPO_4xH_2O$ (276 mg, 2 mmol) was added followed by addition of methyl iodide (903 µl, 14.5 mmol). The mixture was vigorously stirred overnight and then filtered through Celite. The DMF of the filtrate was then rapidly removed under reduced pressure on a rotavapor at 30 °C with oil pump. The residue was then dissolved in dry MeCN (20 ml) and filtered through $MgSO_4$ to remove the residual solid particles. Diisopropylethylamine (1.74 ml, 10 mmol) was then added to the deep orange colored MeCN solution, which immediately turned light yellow. After stirring for 10 min, TLC (A) revealed a new spot with lower R_f (= 0.12) compared to the spot corresponding to the iodide salt (R_f = 0.21). The solid particles that appeared were filtered off through $MgSO_4$ and the filtrate was concentrated. The residue obtained was dissolved in CH_2Cl_2 (20 ml) and extracted with water (2 x 20 ml) in a 50 ml Falcon Tube. The CH_2Cl_2 phase was dried through $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel column chromatography (2 - 8% EtOH in CH_2Cl_2), which afforded pure 2-*N*-(4,4'-dimethoxytrityl)-*N*-7-methyl-2',3'-*O*-methoxymethyleneguanosine Inner Salt (100 mg, 0.117 mmol, 79%), R_f: 0.12 (A). ^{1}H-NMR (270 MHz, $CDCl_3$ + CD_3OD): 9.28 (s, 1H) H8; 7.37-6.76 (m, 13H) arom.; 5.73 (m, 1H) H1'; 5.70 (s, 1H) CH of methoxymethylene; 4.91-4.77 (m, 2H) H2', H3'; 4.09 (s, 3H) *N*-7-Me; 4.01 (m, 1H) H4'; 3.76 (m, 2H) H5',5"; 3.75 (s, 6H) CH_3 of dimethoxytrityl; 3.26 (s, 3H) CH_3 of methoxymethylene.

P^1-(*N*-7-methylguanosine 5')-P^3-(adenosine 5')-1-thiotriphosphate (47), 5'-diphosphoryl- adenosine-2'-(thiophosphoryl)-5'-(*N*-7-methyl)guanosine (48) & 5'-diphosphoryladenosine-3'-(thiophosphoryl) -5'-(*N*-7-methyl)guanosine (49) : The benzoxazaphosphole **23** (90 mg, 0.257 mmol) dissolved in dry CH_2Cl_2 (1.13 ml) was activated under argon by a dry 1M solution of MAC in CH_2Cl_2 (564 µl, 0.564 mmol) during 2h to form the protonated *N*-methylanilino derivative **26A**. The inner salt of 2-*N*-(4,4'-dimethoxy)trityl-*N*-7-methyl-2', 3'-*O*-methoxymethyleneguanosine (148 mg, 0.231 mmol) was coevaporated twice with dry dioxane and dissolved in dry CH_2Cl_2 (820 µl). This solution was added dropwise with a syringe to the solution of **26A** and the resulting reaction solution was stirred for 3 h 30 min. Then this solution was added dropwise over a period of 50 min to a vigorously stirred dry DMF solution of the tri-n-butylammonium salt of ADP (226 mg, 0.283 mmol) and tri-n-butylamine (256 µl, 1.08 mmol). The resulting mixture was stirred for 30 min. Then sublimed sulfur (41 mg, 2.57 mmol) was added and the mixture was stirred for 30 min. Then water (8 ml) was added and the mixture was stirred for another 30 min. The solvents were then evaporated and water (20 ml) was added to the residue and the mixture was extracted with diethyl ether (2 x 20 ml) in a 50 ml Falcon Tube. The aqueous phase was evaporated and the residue was dissolved in 80% aq. AcOH (30 ml) and the solution was stirred for 18 h. The volatile matters were evaporated and the water-diethyl ether extraction was repeated. The residue obtained from evaporation of the aqueous phase was applied to a DEAE-Sephadex A-25 column (2 x 25 cm, HCO_3^- form) and a linear gradient 0.001 M - 0.4 M - 0.5 M of NH_4HCO_3 solution (500 ml / 1000 ml / 500 ml respectively; pH 7.5) was used. The materials were collected in the following manner: The excess ADP eluted between 0.37M to 0.41M. A shoulder peak eluted between 0.41M to 0.43M and was collected separately (Fraction A). A second shoulder eluted between 0.43 M and 0.46 M and was collected separately (Fraction B). These two fractions were purified separately by semi-preparative RP-HPLC chromatography. Batches of 15-20 mg of lyophilised material were each dissolved in 900-1000 µl of solvent A in a Eppendorf tube, filtered through 0.45 µm filters and were then injected onto a semi-preparative Spherisorb S5ODS2 column (8 x 250 mm), pre-equilibrated in solvent A. **Fraction A** was purified with following gradient (1 ml / min; mixer volume 23 ml): solvent A (25 min), then a 30 min increase to 40% solvent B. This gradient run gave, *(1)* pure S_P-isomer of **48** (R_t = 22.8 min, 336 A_{260} units, 13.9 mg Et_3NH^+-salt, 6%), *(2)* both diastereomers of **47**, which separated into two peaks with base-line separation (R_t = 31.2 min, 59 A_{260} units, 3 mg Et_3NH^+-salt) & (R_t = 34.7 min, 51 A_{260} units, 1.8 mg, Et_3NH^+-salt). **Fraction B** was purified in the same way and gave,*(1)* another ~36 A_{260} units of each of the diastereomers of **47** was collected (total yield of **47**, 182 A_{260} units, 3.1%), *(2)* pure R_P-isomer of **48** (R_t = 40.6 min, 377 A_{260} units, 17 mg Et_3NH^+-salt, 7.3%) and *(3)* both diastereomers of **49**, which separated into two peaks with base-line separation (R_t = 49.7 min, 92 A_{260} units, 3.8 mg Et_3NH^+-salt, 1.6%) & (R_t = 51.1 min, 198 A_{260} units, 8.3 mg, Et_3NH^+-salt, 3.6%). The purified material was collected, evaporated and then lyophilised several times (~9 x 2 ml) until the TEAA-salt was removed (monitored by ^{1}H-NMR). The diastereomers of **47** were converted to their Na^+ salts through Dowex Na^+-form. **47** (S_P, Na^+-salt): ^{1}H-NMR (500 MHz, D_2O): 8.30 (s, 1H) AH8; 8.10 (s, 1H) AH2; 5.93 (d, $J_{1',2'}$ = 5.9 Hz, 1H) AH1'; 5.78 (d, $J_{1',2'}$ = 3.5 Hz, 1H) GH1'; 4.59 (dd, $J_{2',3'}$ = 5.2 Hz, 1H) AH2'; 4.45 (dd, $J_{3',4'}$ = 3.7 Hz, 1H) AH3'; 4.41 (dd, $J_{2',3'}$ = 4.9 Hz, 1H) GH2'; 4.38 (dd, $J_{3',4'}$ = 5.2 Hz, 1H) GH3'; 4.33-4.22 (m, 6H) AH4', 5', 5" & GH4', 5' ,5"; 3.96 (s, 3H) G*N*-7-Me. **47** (R_P, Na^+-salt): ^{1}H-NMR (500 MHz, D_2O): 8.34 (s, 1H) AH8; 8.08 (s, 1H) AH2; 5.93 (d, $J_{1',2'}$

= 6.2 Hz, 1H) AH1'; 5.77 (d, $J_{1',2'}$ = 2.9 Hz, 1H) GH1'; 4.57 (dd, $J_{2',3'}$ = 5.1 Hz, 1H) AH2'; 4.43 (dd, $J_{3',4'}$ = 3.3 Hz, 1H) AH3'; 4.36 (dd, $J_{2',3'}$ = 4.4 Hz, 1H) GH2'; 4.38 -4.20 (m, 7H) AH4', 5', 5" & GH3', 4', 5' ,5"; 3.95 (s, 3H) G*N*-7-Me. MS (FAB$^-$): calc for (M-Na$^+$) 868.0 found 868.1 **48** (*S*$_P$, Et_3NH^+-salt): ^{1}H-NMR (270 MHz, D_2O): 8.52 (s, 1H) AH8; 8.48 (s, 1H) GH8; 8.10 (s, 1H) AH2; 6.17 (d, $J_{1',2'}$ = 6.6 Hz, 1H) AH1'; 5.71 (d, $J_{1',2'}$ = 1.3 Hz, 1H) GH1'; 5.50 (ddd, $J_{2',3'}$ = 5.6 Hz, $J_{2',P}$ = 12.5 Hz, 1H) AH2'; 4.64 (m, 1H) AH3'; 4.36 (m, 1H) AH4'; 4.18-4.02 (m, 7H) AH5', 5" & GH2',3',4', 5', 5"; 3.93 (s, 3H) G*N*-7-Me; 3.12 (q, 12H) CH_2 of 2 x Et_3NH^+; 1.20 (t, 18H) CH_3 of 2 x Et_3NH^+. **48** (*R*$_P$, Et_3NH^+-salt): ^{1}H-NMR (270 MHz, D_2O): 8.84 (s, 1H) GH8; 8.45 (s, 1H) AH8; 8.05 (s, 1H) AH2; 6.15 (d, $J_{1',2'}$ = 6.5 Hz, 1H) AH1'; 5.72 (d, $J_{1',2'}$ = 2.71 Hz, 1H) GH1'; 5.25 (ddd, $J_{2',3'}$ = 5.3 Hz, $J_{2',P}$ = 10.44 Hz, 1H) AH2'; 4.63 (m, 1H) AH3'; 4.39 (m, 1H) GH2'; 4.34 (m, 1H) AH4'; 4.41-4.05 (m, 6H) AH4', 5', 5" & GH3', 4', 5', 5"; 3.91 (s, 3H) G*N*-7-Me; 3.12 (q, 12H) CH_2 of 2 x Et_3NH^+; 1.20 (t, 18H) CH_3 of 2 x Et_3NH^+ **49** (*S*$_P$, Et_3NH^+-salt): ^{1}H-NMR (270 MHz, D_2O): 9.06 (s, 1H) GH8; 8.48 (s, 1H) AH8; 8.18 (s, 1H) AH2; 5.99 (d, $J_{1',2'}$ = 6.5 Hz, 1H) AH1'; 5.93 (d, $J_{1',2'}$ = 3.9 Hz, 1H) GH1'; 4.94 (m, 1H) AH3'; 4.77 (m, 1H) AH2'; 4.64 (m, 1H) GH2'; 4.55 (m, 1H) AH4'; 4.46 (m, 1H) GH3'; 4.37 (m, 1H) GH4'; 4.30-4.07 (m, 4H) AH5', 5" & GH5', 5"; 3.98 (s, 3H) G*N*-7-Me; 3.12 (q, 12H) CH_2 of 2 x Et_3NH^+; 1.20 (t, 18H) CH_3 of 2 x Et_3NH^+. **49** (*R*$_P$, Et_3NH^+-salt): ^{1}H-NMR (270 MHz, D_2O): 8.98 (s, 1H) GH8; 8.49 (s, 1H) AH8; 8.13 (s, 1H) AH2; 6.01 (d, $J_{1',2'}$ = 5.6 Hz, 1H) AH1'; 5.99 (d, $J_{1',2'}$ = 3.2 Hz, 1H) GH1'; 4.85-4.75 (m, 2H) AH2',3'; 4.57 (m, 2H) GH2', AH4'; 4.44 (m, 1H) GH3'; 4.35 (m, 1H) GH4'; 4.28-4.10 (m, 4H) AH5', 5" & GH5', 5"; 3.97 (s, 3H) G*N*-7-Me; 3.12 (q, 12H) CH_2 of 2 x Et_3NH^+; 1.20 (t, 18H) CH_3 of 2 x Et_3NH^+.

ACKNOWLEDGEMENTS

The authors thank the Swedish Board for Technical Development (NUTEK) and Swedish Natural Science Research Council for generous financial support. Fund for the purchase of a 500 MHz NMR spectrometer from Wallenbergs Stifelsen, University of Uppsala and Swedish Research Council (FRN) is gratefully acknowledged. The authors also acknowledge the financial support by Deutsche Forschungsgemeinschaft and Fonds der chemischen Industrie. The authors also thank Mr. Christhophe Thibaudeau, Dr. Peter Agback and Mr. Janez Plavec for recording 1D and 2D ^{1}H-NMR at 500 MHz and ^{31}P-NMR at 202 MHz.

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(*Received in UK* 28 *November* 1994; *revised* 4 *January* 1995; *accepted* 6 *January* 1995)