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A CONVERGENT REGIOSPECIFIC SYNTHESIS OF THE LARIAT-TRINUCLEOTIDES $A^{2'p5'G}_{3'p5'U}$ AND $A^{2'p5'G}_{3'p5'C}$ FROM A O^4-(2-NITROPHENYL)-URIDINE BUILDING BLOCK.

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Abstract: Total synthesis of title compounds 1 and 2 from a common intermediate 7 is reported using the phosphotriester-phosphiteamidite approach. Appropriate NMR evidence has been presented in support of the regiospecific synthesis of target molecules in addition to enzymatic analysis. Present work clearly shows that the NMR evidence is mandatory to establish the isomeric purity of branched RNA molecules; enzymatic or/and electrophoretic analysis alone as tools for confirmation of branched RNA structures can be misleading.

Recent studies[1-4] have elegantly demonstrated that a significant aspect of pre-mRNA processing (splicing) in eucaryotic cell is mediated by either of the two branched trinucleotides, 1 and 2, - the intron "lariats". It is apparent from the literature that the availability of specific pure lariats in large quantity will significantly aid in the biological studies to elucidate its role in the order of intron excision from pre-mRNA, its role in duplex formation during splicing and also in understanding of the energetics during splicing. Two efforts[5,6] have been already reported in the literature. Herein we report our studies on the regiospecific synthesis of the trinucleotides 1 and 2 using first a phosphotriester approach to build the dimer 3 and then selectively phosphorylate the 2'-hydroxyl function of the partially protected dinucleotide 4 with 5'-phosphoramidite of guanosine block 6 to give the partially protected trimer 7 which then gives either lariat 1 or 2 selectively, depending upon a precise deprotection condition.

It has been clearly shown[7,8] that any attempt to remove the 2'-acid labile group from the fully protected trimer, as in 3, caused a considerable break-down

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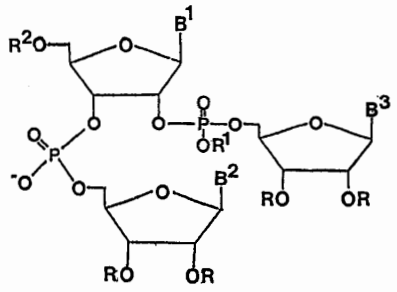
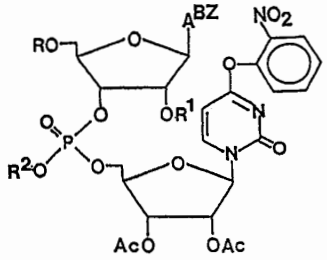
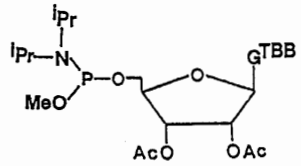


1; B^1 = 9-ADENINYL; B^2 = 1-URACILYL; B^3 = 9-GUANINYL; R = R^1 = R^2 = H

2; B^1 = 9-ADENINYL; B^2 = 1-CYTOSINYL; B^3 = 9-GUANINYL; R = R^1 = R^2 = H

7; B^1 = A^{Bz}; B^2 = O^4-(2-NITROPHENYL)-2-PYRIDONE-1-YL; B^3 = G^{Tbb}
R = Ac; R^1 = Me; R^2 = Toluoyl (Tol)





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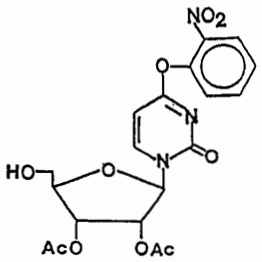
3; R = 4-TOLUOYL (Tol); R^1 = 9-(PHENYLXANTHEN)-9-YL (Pixyl)

4; R = Tol; R^1 = R^2 = H

5; R = Tol; R^1 = Pixyl; R^2 = H



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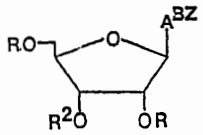
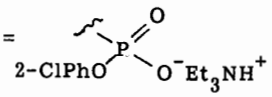


8; R = Tol; R^1 = Pixyl; R^2 = H

9; R = Tol; R^1 = Pixyl; R^2 = 

A^{Bz} = N^6-BENZOYL-9-ADENINYL; G^{Tbb} = N^2-(4-t-BUTYL)BENZOYL-9-GUANINYL

to mononucleotides and nucleosides and isomerization of the 3'→5' internucleotide linkage to 2'→5' and vice versa. This was found to be due to the participation of the 2'-hydroxyl group in a transesterification reaction with the neighbouring phosphotriester. On the other hand, it is also clearly demonstrated[9] that one may safely remove the 2'-acid labile group from a 3'→5' phosphodiester as in 5 with help of 80% aq. acetic acid at 20 °C. We thus prepared the fully protected dinucleotide 3 in 90% yield by the condensation of the 5'-protected phosphodiester block 9, prepared from 8, and the 5'-hydroxy block 10 in the presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole[10] using standard procedures developed in the phosphotriester chemistry[11-15]. The pyrimidine block 10 was protected with the 2-nitrophenyl group at the O^4 position[16]. This was considered essential[17,18] because such an intermediate, at the end of the synthesis of the target molecule, could be converted either to cytosine residue or to uracil moiety, depending upon the order of use of either a oxygen or nitrogen nucleophile in the final deprotection stage. The 2-chlorophenyl group from the internucleotide phosphate of 3 was smoothly removed to give the 3'→ 5' phosphodiester 5 with tetrabutylammonium fluoride (2 eq.) in THF- pyridine-water mixture (8:1:1, v/v/v, 4 h, 20 °C)[19], followed by the removal of the volatile matters in vacuo and coevaporation with ethanol. Subsequently, the 2'-acid labile group[24] from 5 was removed by the treatment with 80% aqueous acetic acid[9] at 20 °C for 20 min followed by the removal of volatile matters by coevaporation with dioxane to give partially protected dinucleotide 4. A ^1H- and ^{31}P NMR examination of crude 4 clearly revealed that (i) the 2-nitrophenyl group at O^4 was intact, (ii) the internucleotide 2-chlorophenyl and 2'-O-(9-phenylxanthen-9-yl)[24] groups were removed selectively and (iii) the specific 3'→ 5' phosphodiester linkage is unaltered. The partially protected dinucleotide 4 was dried carefully over P_2O_5 in vacuo. A mixture of 4 (0.22 mmol) and the 5'-phosphoramidite 6 (5 eq.) was then reacted in dry acetonitrile (10 ml/mmol), in presence of freshly sublimed tetrazole (50 eq.)[20], under an atmosphere of argon for 1 h at 20 °C, followed by a work-up and oxidation with iodine-water mixture[20,21] to give the protected trimer 7 which was purified on a short silica gel column (eluent: 20% methanol in chloroform). The trimer 7 was subsequently deprotected in two different ways[17,18] to give either 1 or 2 depending upon the exact deprotection condition: (1) syn 4-nitrobenzaldoximate ion[10] in dioxane-water mixture for 24 h at 20 °C followed by aqueous ammonia treatment for 5 days at 20 °C and separation of the crude mixture on a DEAE Sephadex A25 column gave 1 (36%); (2) liquid ammonia treatment for 48 h followed by aqueous ammonia for 5 days at 20 °C gave the lariat 2 after a standard work up and purification through a DEAE Sephadex A25 column (52%). The elution profiles are shown in Fig. 1 and 2 respectively. The HPLC purity of products 1 and 2 are shown in panels A and B respectively in Fig. 3, while panel C in Fig. 3 shows the separation of a ca. 7:3 artificial mixture of 1 and 2. The fully deprotected lariats 1 and 2 were completely stable for 48 h in 0.1 M aq. NaOH and to the

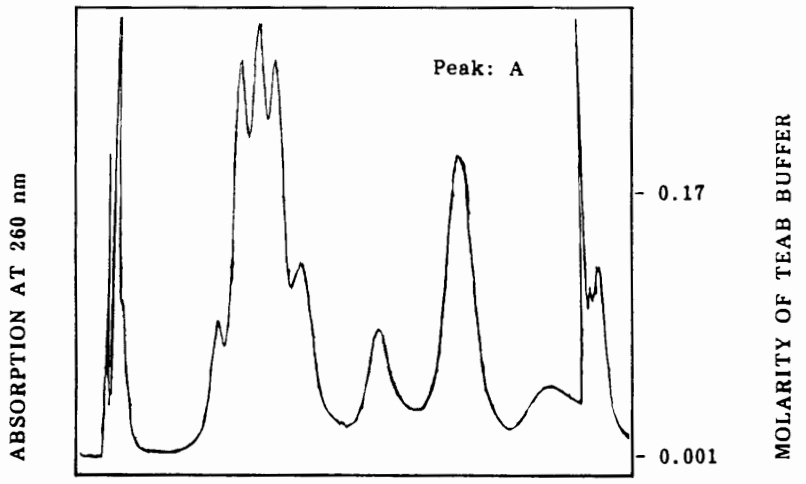


FIGURE 1 : PURIFICATION OF $A^{2'p5'G}_{3'p5'U}$ (1) (peak: A) ON A DEAE SEPHADEX A25 COLUMN.

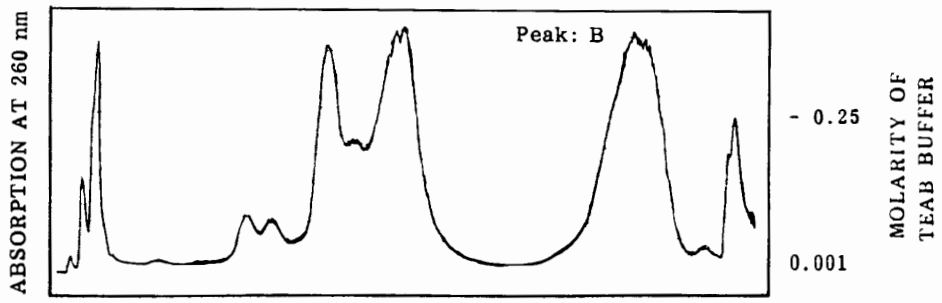


FIGURE 2 : PURIFICATION OF $A^{2'p5'G}_{3'p5'C}$ (2) (peak: B) ON A DEAE SEPHADEX A25 COLUMN.

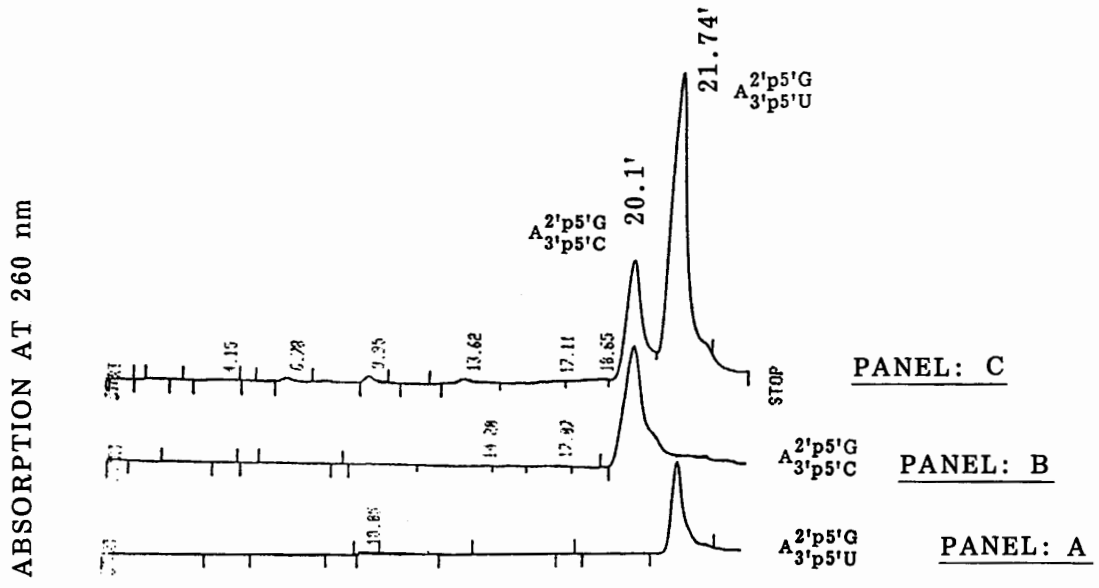


FIGURE 3 : HPLC PURITIES OF COMPOUNDS 1 & 2 (panels: A & B) AND A SEPARATION OF THEIR ARTIFICIAL MIXTURE (panel: C)

treatment of spleen phosphodiesterase. Lariat 1 was degraded to 5'-UMP, 5'-GMP and adenosine and 2 to 5'-CMP,5'-GMP and adenosine, in equimolar ratios, upon treatment with snake venom phosphodiesterase. The 1D 270 MHz ^{1}H-NMR spectrum of 1 and 2 are shown in Figs. 4 and 5 respectively establishing their isomeric purities. The 2D COSY spectra and Hartmann-Hahn NMR spectra[26] of 1 and 2 are shown in Figs. 6 and 8 and 7 and 9 respectively which assigned the proton resonances of target molecules as detailed in Table 1. The UV and ^{31}P-NMR spectra of 1 and 2 are also shown in Figs. 11, 12 and 13 respectively.

While this work was in progress Caruthers et al.[22] reported a similar strategy with the t-butyldimethylsilyl group for the protection of 2'-hydroxyl function which is known to isomerize to the 3'-hydroxyl function under mild basic condition[25]. It should be noted that none of the three procedures reported so far in the literature[5,6,22] gives any spectroscopic evidence on the isomeric purity of the lariat structures despite the fact that these workers[6,22] have employed either a 2' or 3' protecting group which have potential to isomerize to the vicinal hydroxyl function. It is clear that the evidence of regioselective synthesis of any lariat molecules, either 1 or 2, by simple enzymatic digestion and electrophoretic mobility can not be considered as conclusive since any isomeric molecule formed during the synthesis[6,22] will also be expected to give similar results. We believe that a ^{1}H-NMR analysis of the fully deprotected lariats can definitely indicate the extent of isomerization as exemplified in Fig. 10 which shows a mixture of $A^{2'p5'C}_{3'p5'G}$ and $A^{2'p5'G}_{3'p5'C}$ that were formed due to the partial isomerization of 3'→5' phosphodiester of 4 to the corresponding 2'→5' isomer at the initial stages of the present work.

Experimental

^{1}H-NMR spectra were recorded, in δ scale, at 90 MHz and 270 MHz with Jeol FX 90Q and Jeol JNM-GX 270 spectrometers, TMS or acetonitrile (set at 2 ppm) being used as an internal standard. ^{31}P-NMR spectra were recorded at 36 MHz in the same solvent as for ^{1}H-NMR using phosphoric acid as an external standard (δ scale). Details regarding the NMR spectroscopic measurements will be reported elsewhere. UV spectra were measured using a Cary/Varian 2200 spectrometer. TLC was carried out using pre-coated silica gel F_{254} plates in the following solvent systems: (A) ethanol-dichloromethane 9.5:0.5, v/v. (B) ethanol-dichloromethane 9:1, v/v. The short column chromatographic separations were carried out using Merck G60 silica gel. HPLC was performed[23] using sepherisorb (10 μ) ODS column (20 cm x 4 mm) using solvent A: 5 x 10^{-4} M tetrapentylammonium-phosphate (TPAP). Solvent B: 5 x 10^{-4} M TPAP in 20% acetonitrile-water at pH 7 at 20 °C.

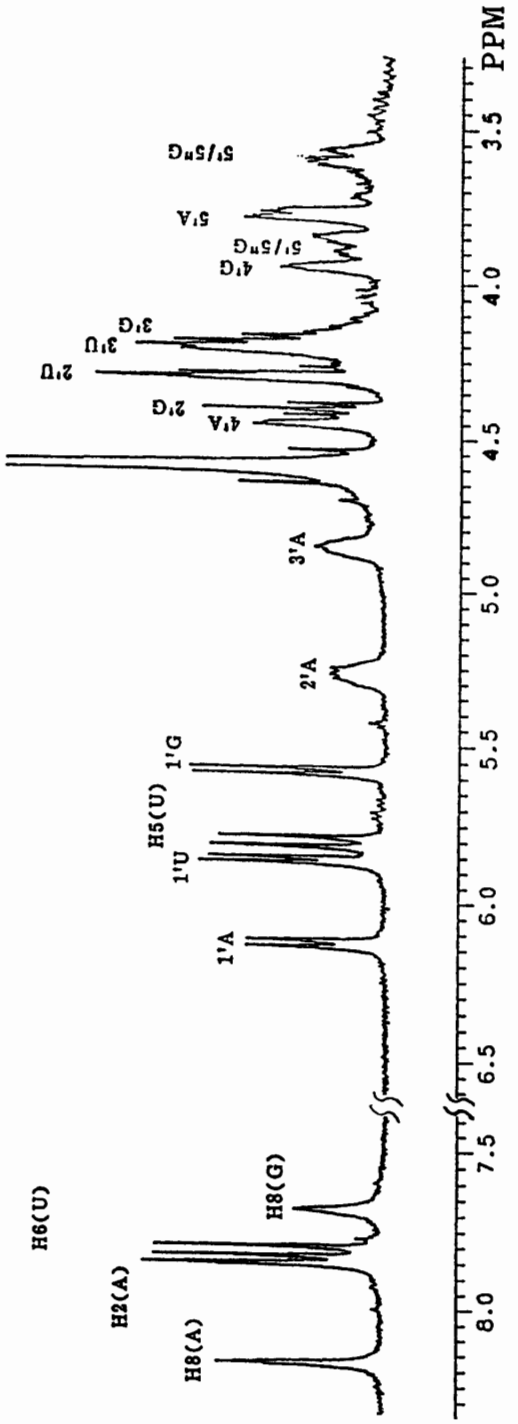


FIG. 4 : 270 MHz ^{1}H-NMR SPECTRUM OF 1

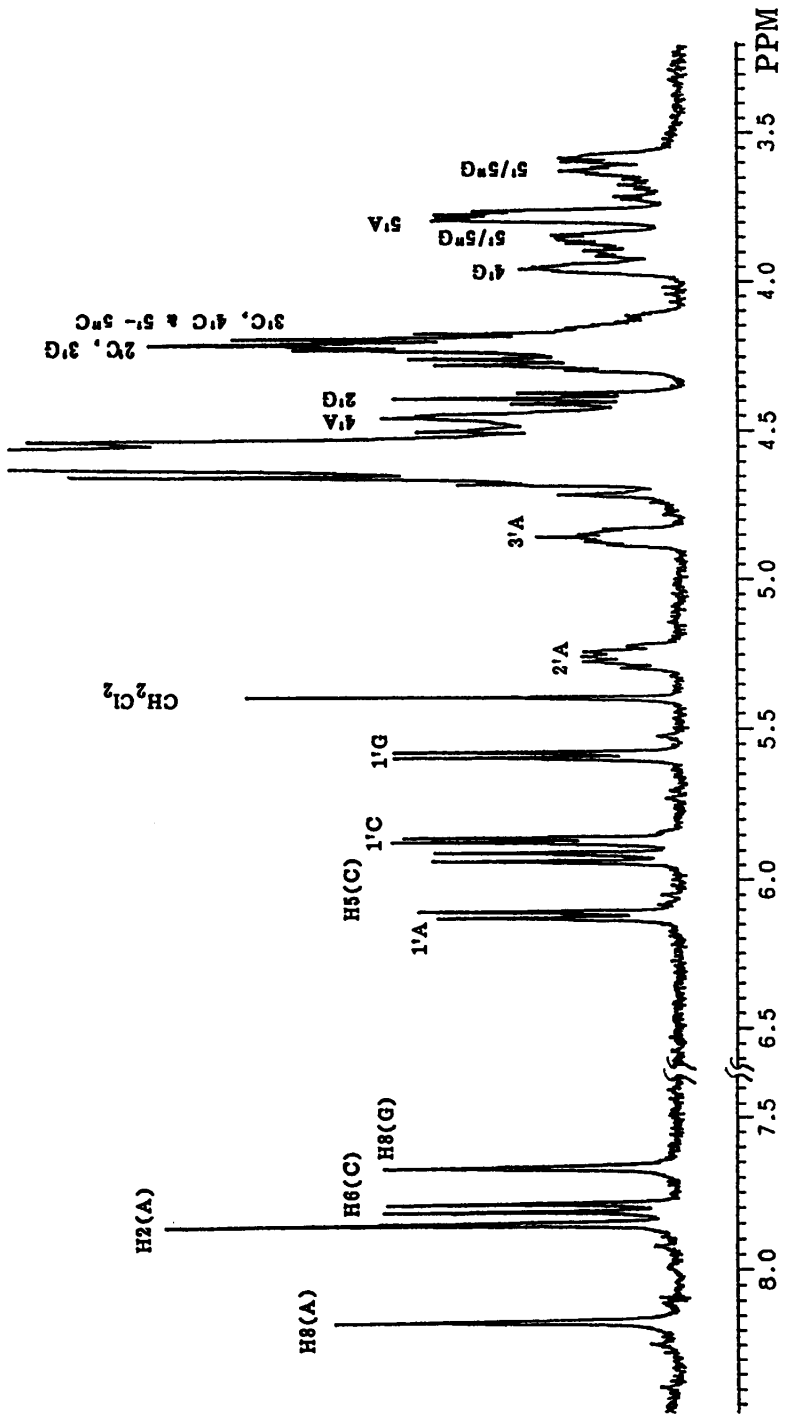


FIG. 5 : 270 MHz ^{1}H-NMR SPECTRUM OF 2

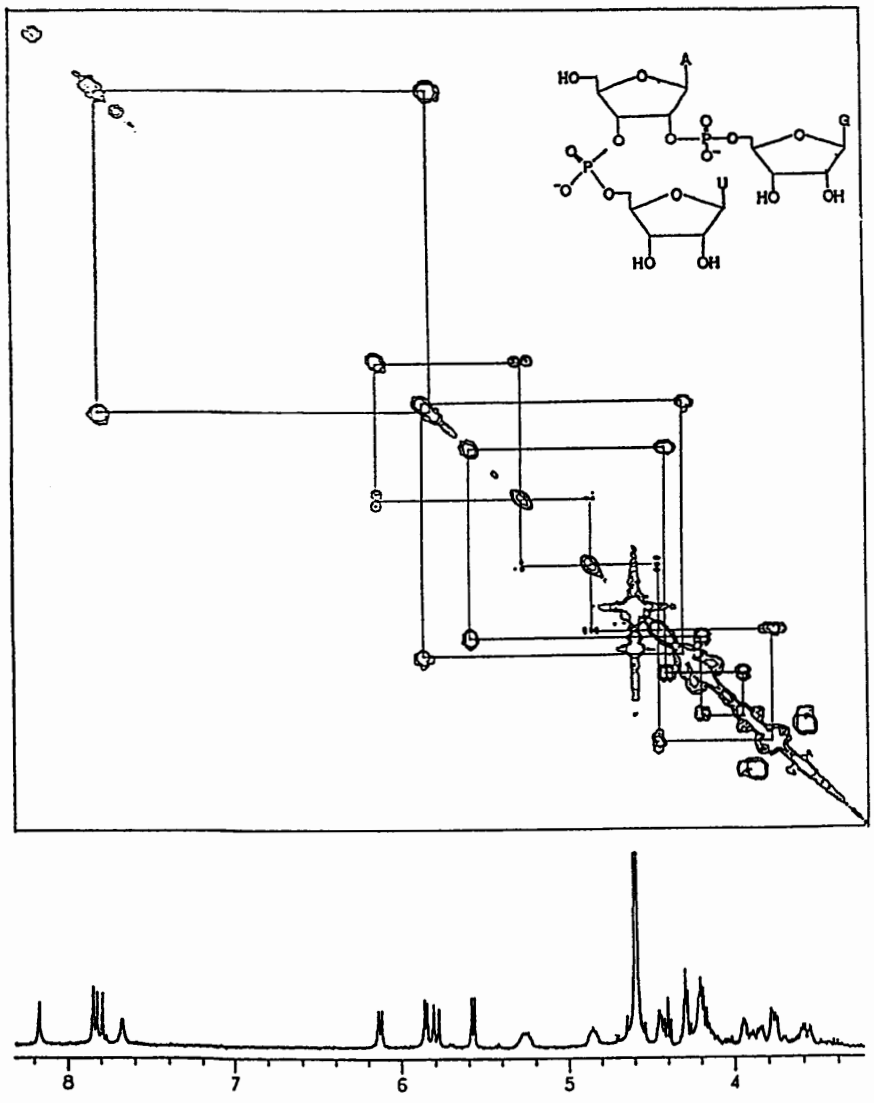


FIG. 6 : 2D COSY SPECTRUM OF $A^{2'p5'G}_{3'p5'U}$ (1)

Synthesis of O^4-(2-nitrophenyl)-2',3'-di-O-acetyluridine (10). To a solution of 5'-dimethoxytrityl-2',3'-di-O-acetyluridine (1 mmol, 0.64 g) in dichloromethane (10 ml) was added 1-mesitylenesulphonyl chloride (0.61 g, 3 mmol), triethylamine (2.3 ml, 15 mmol), N,N-dimethylaminopyridine (12 mg, 0.1 mmol) and the reaction stirred for 1 h. DABCO (0.11 g, 1 mmol), 2-nitrophenol (2.3 ml, 5 mmol) was then added and the reaction stirred for 30 min at 20 °C. The reaction mixture was then poured into 0.5 M citric acid solution (200 ml) and extracted with dichloromethane (3 x 60 ml). The combined extracts were washed with water (2 x 100 ml) and

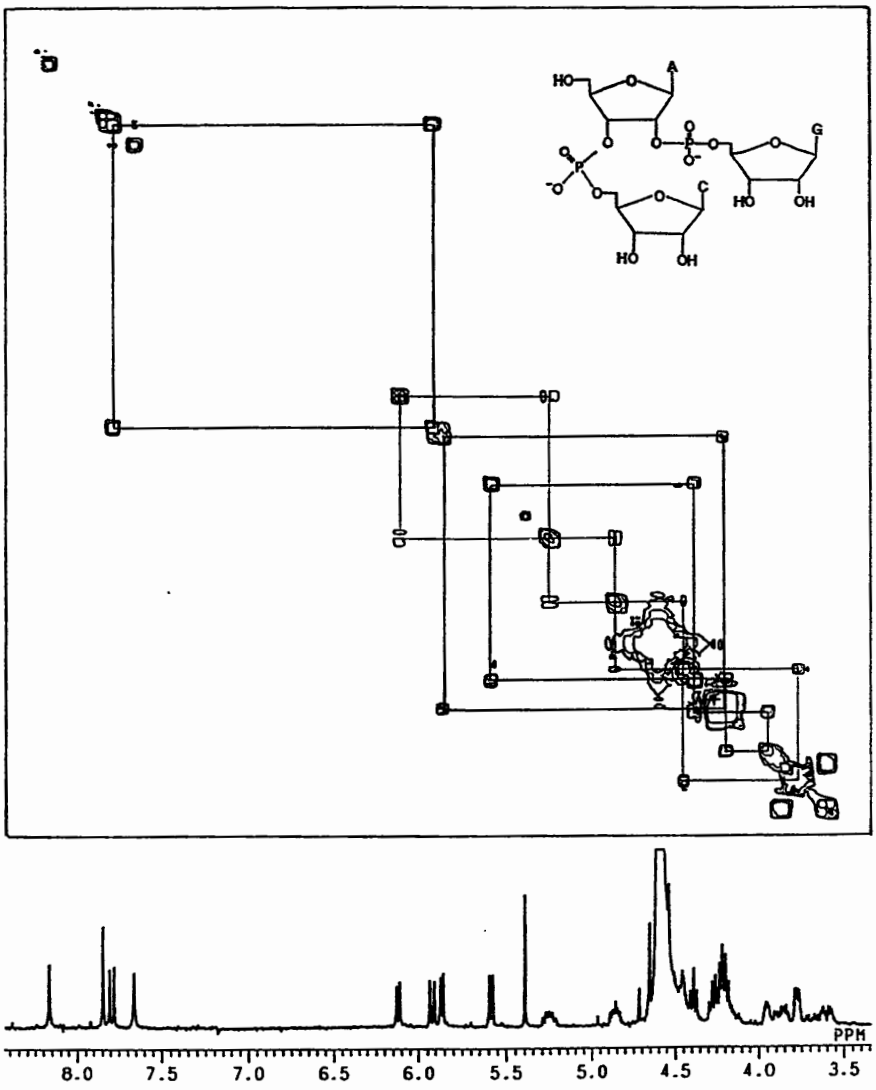


FIG. 7 : 2D COSY SPECTRUM OF $A^{2'p5'G}_{3'p5'C}$ (2)

evaporated (R_f 0.79, solvent: B). The resultant residue was dissolved in 10% methanol-dichlormethane mixture (10 ml) and 4-toluenesulphonic acid monohydrate (0.95 g, 5 mmol) in 10% methanol-dichloromethane mixture (10 ml) was added and the reaction stirred for 5 min. The reaction mixture was then poured into saturated sodium hydrogen carbonate (100 ml) and extracted with dichloromethane (2 x 100 ml). The combined extracts were evaporated, and purified by silica gel chromatography. The desired compound was eluted with 2% ethanol-dichloromethane mixture. Appropriate fractions were evaporated and finally precipitated and dried

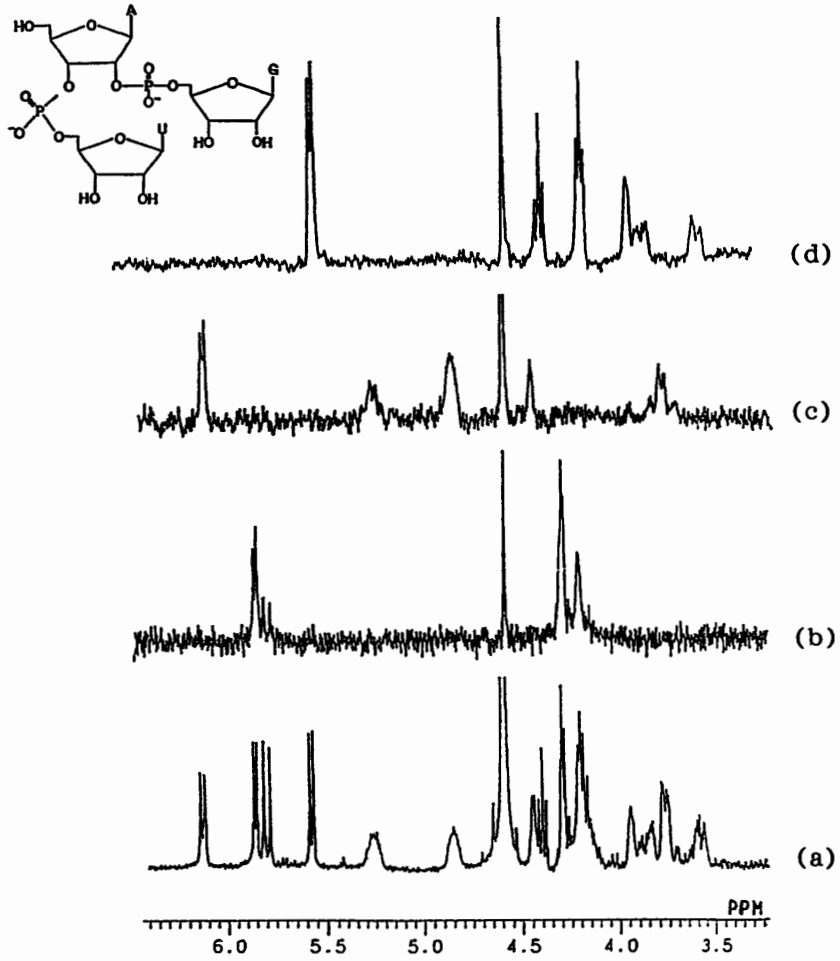


FIG. 8 : 270 MHz SPECTRA OF THE RIBOSE REGION OF 1

(a) Regular spectrum;

(b-d) Selective excitation subspectra of U5'-p, $A^{2'-p}_{3'-p}$ and G5'-p.

(0.44 g, 98%). R_f: 0.64 (solvent: B). ^{1}H-NMR ($CDCl_3$ + CD_3OD): 8.27 (d, 7.3 Hz, 1H) H-6 of pyrimidone; 8.14 (d, 9.1 Hz, 1H) H-2 of O^4-(2-nitrophenyl); 7.77-7.27 (m, 3H) 2-nitrophenyl; 6.29 (d, 7.3 Hz, 1H) H-5; 6.13 (d, 4.9 Hz, 1H) H-1'; 5.48 (m, 2H) H-2' and -3'; 4.24 (m, 1H) H-4'; 3.85 (m, 2H) H-5'; 2.12 (s, 3H) acetate; 2.08 (s, 3H) acetate.

N^6-Benzoyl-2'-O-(9-phenylxanthen-9-yl)(pixyl)-5'-O-toluoyladenosine (8). N^6-benzoyl-2'-pixyladenosine[24] (1 mmol) was dissolved in dry pyridine

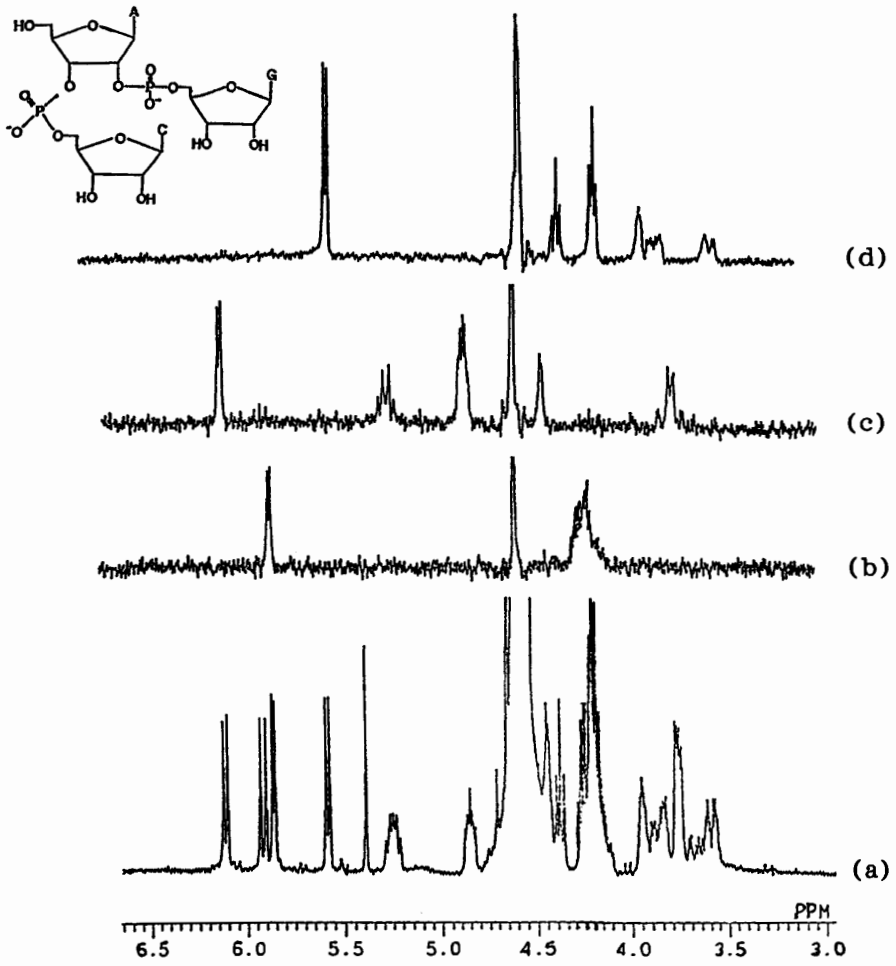


FIG. 9 : 270 MHz SPECTRA OF THE RIBOSE OF 2

(a) Regular spectrum;

(b-d) Selective excitation subspectra of C5'-p, $A^{2'-p}_{3'-p}$ and G5'-p.

(15 ml) and evaporated to dryness to remove any moisture. The residue was dissolved in dry pyridine (10 ml), and toluoyl chloride (1.1 mmol) was slowly added via a syringe under dry conditions. After 45 min, the reaction was found to be complete by tlc (R_f: 0.33, solvent: A). Methanol (1 ml) was now added and the reaction mixture stirred for 30 min and then evaporated. The residue was dissolved in dichloromethane (100 ml) and poured into saturated sodium hydrogen car-

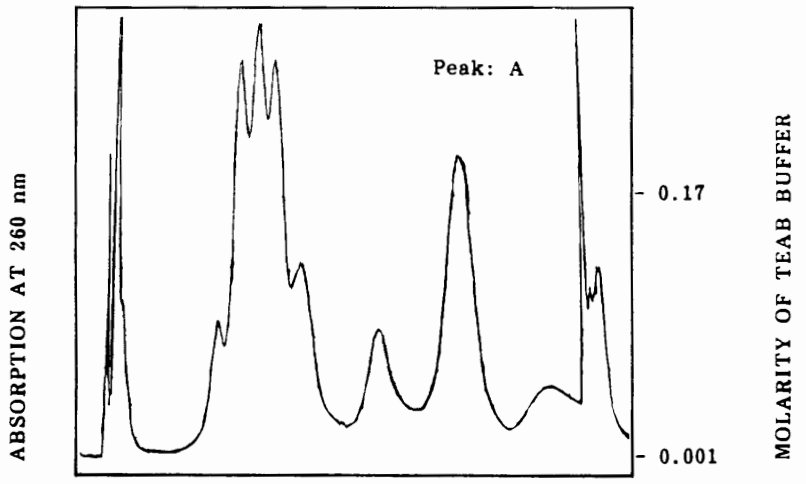


FIGURE 1 : PURIFICATION OF $A^{2'p5'G}_{3'p5'U}$ (1) (peak: A) ON A DEAE SEPHADEX A25 COLUMN.

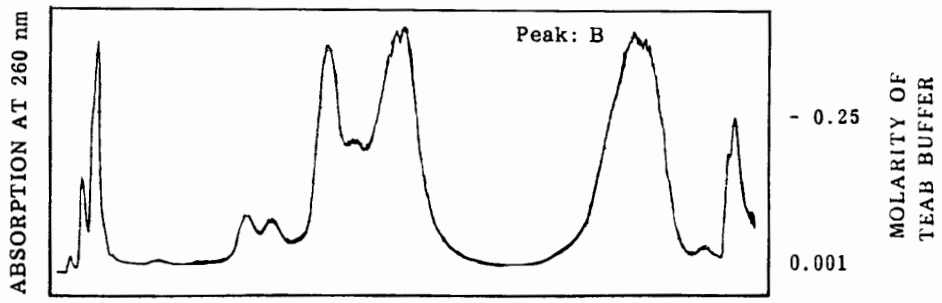


FIGURE 2 : PURIFICATION OF $A^{2'p5'G}_{3'p5'C}$ (2) (peak: B) ON A DEAE SEPHADEX A25 COLUMN.

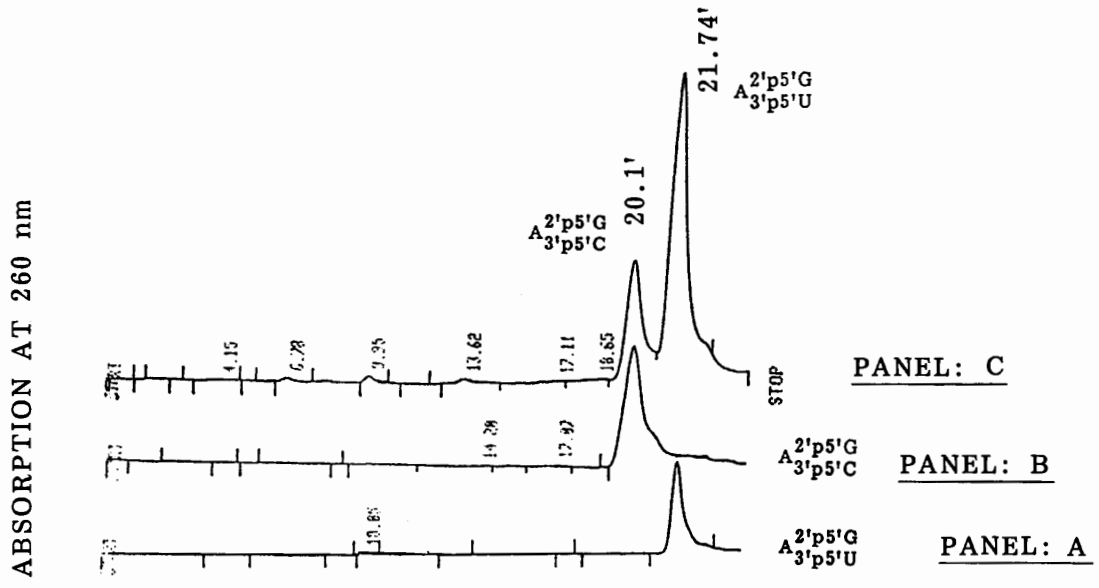


FIGURE 3 : HPLC PURITIES OF COMPOUNDS 1 & 2 (panels: A & B) AND A SEPARATION OF THEIR ARTIFICIAL MIXTURE (panel: C)

Table 1: ^{1}H-NMR chemical shifts recorded at 270 MHz in D_2O (CH_3CN as internal standard set at 2.0 ppm; accuracy of chemical shifts ±0.01 ppm) of compounds 1 and 2.

Compound 1	H_8	H_2	H_6	H_5	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$
A	8.17	7.85	-	-	6.13	5.26	4.85	4.45	3.81	3.73
U	-	-	7.81	5.80	5.86	4.30	4.20		4.12	
G	7.67	-	-	-	5.57	4.40	4.19	3.94	3.87	3.58
Compound 2										
A	8.16	7.84	-	-	6.11	5.25	4.85	4.45	3.81	3.73
C	-	-	7.79	5.92	5.86	4.27	4.21		4.13	
G	7.66	-	-	-	5.58	4.39	4.19	3.95	3.88	3.60

bonate solution (100 ml) and extracted. The dichloromethane layer was run off, and the aqueous layer washed with dichloromethane (3 x 100 ml). The combined extracts were coevaporated with toluene and the residue purified by silica gel chromatography. The desired product was eluted with 1% methanol-dichloromethane mixture containig 1% pyridine, was evaporated and precipitated from hexane and dried (731 mg, 98%). ^{1}H-NMR ($CDCl_3$ + Dabco): 8.3 (s, 1H) H-8; 8.0-6.8 (m, 23H) arom; 5.95 (d, 7.3 Hz, 1H) H-1'; 4.90 (dd, 7.3 and 5.2 Hz, 1H) H-2'; 4.55-4.19 (m, 3H) H-4' and 5'; 3.39 (d, 5.2 Hz, 1H) H-3'; 2.85 (s, 3H) methyl of toluoyl.

3'-O-(2-chlorophenyl)phosphoro-N^6-benzoyl-2'-O-pixyladenosine (9). Compound 8 (1 mmol) was dissolved in dry pyridine (8 ml) and a solution of o-chlorophenyl-phosphoro-bis-(1,2,4-triazolide) in acetonitrile (8 ml, 2 mmol) was added and the reaction stirred for 20 min and a tlc examination showed the disappearence of starting amterial (R_f 0.58, solvent: B) and a new product (R_f 0.13, solvent: B) had been formed. The reaction mixture was then poured into 0.2 M triethylammonium bicarbonate solution (pH 7.2) solution (100 ml) and extracted with dichloromethane (4 x 100 ml). The combined dichloromethane extracts was washed with water (100 ml), and coevaporated with toluene. The residue was then dissolved in dichloromethane (5 ml) and precipitated from hexane (50 ml), centrifuged and dried (0.95 g, 95%). ^{1}H-NMR ($CDCl_3$ + Dabco): 8.3 (s, 1H) H-8; 8.1-6.8 (m, 27H) arom; 6.14 (d, 8 Hz, 1H) H-1'; 5.19 (m, 1H) H-2'; 4.80 (m, 1H) H-3'; 4.43 (m, 3H) H-4' and 5'; 2.84 (s, 3H) methyl of toluoyl. ^{31}P-NMR ($CDCl_3$ + Dabco): -5.9 (sole signal in ^{31}P-NMR).

Dimer block (3). The phosphodiester (9) (1.1 g, 1.1 mmol) and O^4-(2-nitrophenyl)-2',3'-di-O-acetyluridine (0.44 g, 1 mmol) were dissolved in dry pyridine

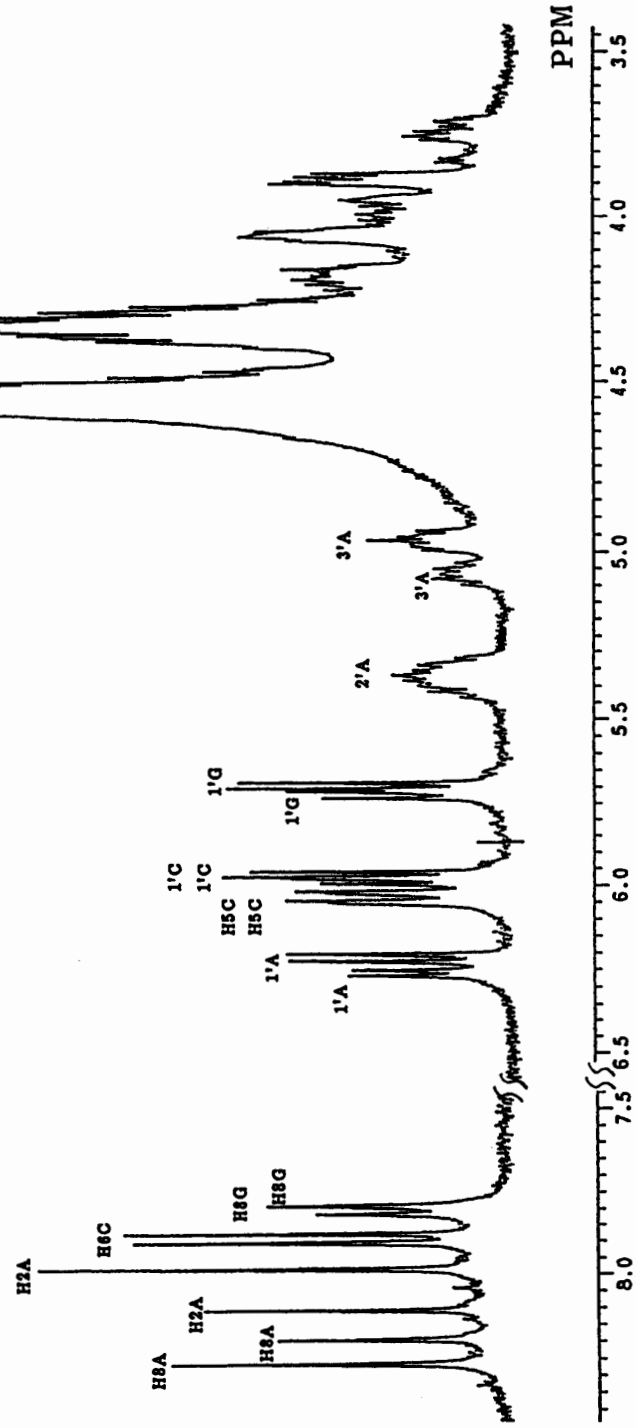
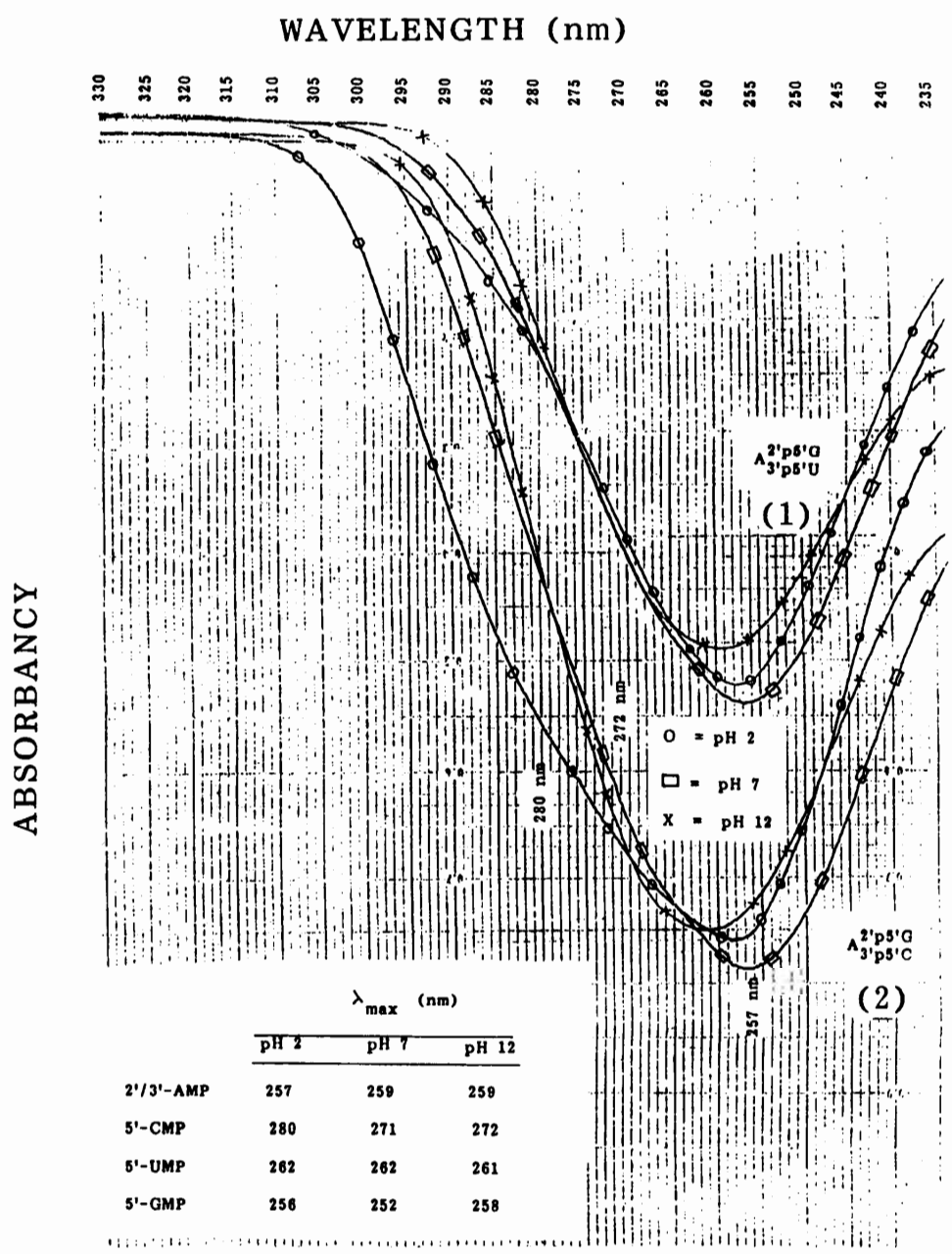


FIG. 10 : 270 MHz ^{1}H-NMR SPECTRUM OF A MIXTURE OF $A^{2'p5'G}_{3'p5'C}$ & $A^{2'p5'C}_{3'p5'G}$



λ_{max} (nm)

	pH 2	pH 7	pH 12
2'/3'-AMP	257	259	259
5'-CMP	280	271	272
5'-UMP	262	262	261
5'-GMP	256	252	258

FIG. 11 : THE UV SPECTRA OF LARIATS 1 & 2 (Note the shift of 2 in comparison with 1 at pH 2). Inset Table shows some literature values (cf. Handbook of Biochem. & Mol. Biol., CRC Press, 3rd. ed., 1975).

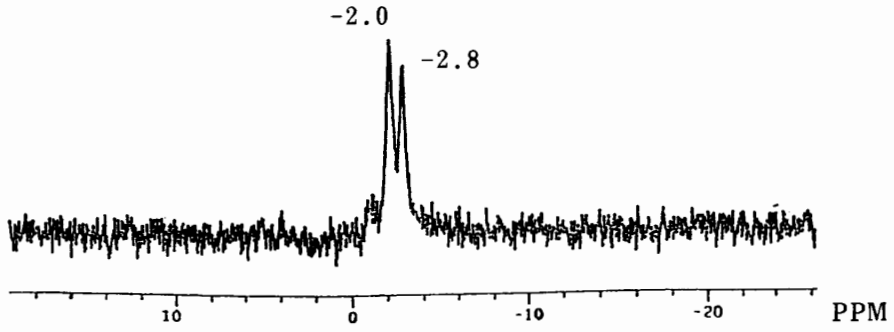


FIG. 12 : ^{31}P-NMR (D_2O) OF LARIAT 1

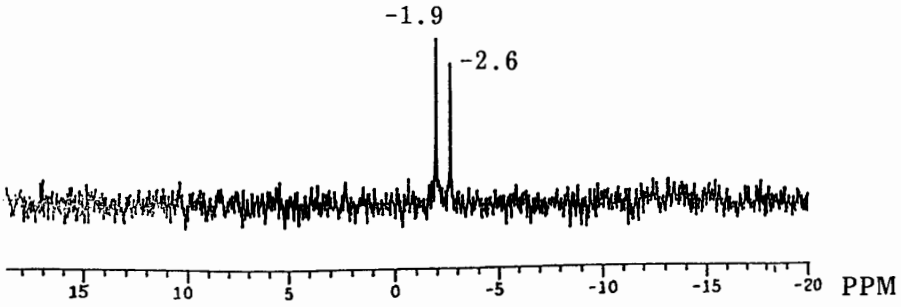


FIG. 13 : ^{31}P-NMR (D_2O) OF LARIAT 2

(5 ml), and evaporated to dryness. The residue was dissolved in dry pyridine (10 ml) and 1-mesitylenesulfonyl-3-nitro- (1,2,4-triazole) added and the reaction stirred for 30 min and examined by tlc. All of the uridine block had been consumed and a new product (R_f 0.76, solvent: A) had been formed. The reaction mixture was then poured into saturated sodium hydrogen carbonate solution (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined extracts were evaporated and coevaporated with toluene, and purified by silica gel chromatography, using 1% pyridine-dichloromethane mixture. The product was collected, evaporated and precipitated from hexane, centrifuged and dried. (1.24 g, 93%). ^{31}P-NMR ($CDCl_3$): -7.68 and -8.30 (only signals from a pair of diastereoisomers of 3).

Deprotection of Dimer block 3 to give 4. The fully protected dimer 3 (1.3 g, 1 mmol) was dissolved in tetrahydrofuran:pyridine:water (38 ml, 8:1:1 v/v/v) and tetrabutylammonium fluoride in THF (2 ml, 1 M solution) was added and the reaction stirred for 4 h at 20 °C. The volatile matters were then removed and the

residue was purified by silica gel chromatography. The column was washed with 1% pyridine-dichloromethane mixture and the product was eluted with 8% ethanol in the same solvent mixture. The desired fractions were pooled and evaporated then coevaporated with toluene. The pyridine free residue of 5, thus obtained, was dissolved in 80% acetic acid (15 ml) and stirred for 20 min at 20 °C. The volatile matters were removed, and the residue dissolved in dioxane and evaporated. This step was repeated twice more to ensure that all of the acetic acid was removed. The residue was dissolved in dichloromethane (5 ml) and precipitated from diethyl ether, centrifuged and dried to give 4 (1.0 g, 88%, based on 3). ^{1}H-NMR ($CDCl_3$): 8.7 (s, 1H) H-8; 8.67 (d, 5.4 Hz, 1H) H-3 of O^4-(2-nitrophenyl); 8.21 (s, 1H) H-2; 8.14-7.1 (m, 13H) arom; 6.45 (d, 7.6 Hz, 1H) H-5; 6.35 (d, 5.1 Hz, 1H) H-1'; 6.24 (d, 5.4 Hz, 1H) H-5 of pyrimidone; 5.49 (m, 2H) H-2'' and -3''; 5.07 (m, 2H) H-2' and 3'; 4.65 (m, 3H) H-4'' and 5''; 4.33 (m, 3H) H-4' and H-5'; 3.25 (m, 6H) -CH_2 of triethyl ammonium; 2.38 (s, 3H) methyl of toluoyl; 2.08 (s, 3H) acetate; 2.0 (s, 3H) acetate; 1.4 (m, 9H) methyl of triethylammonium. ^{31}P-NMR ($CDCl_3$): +1.07 (sole signal in ^{31}P-NMR).

Synthesis of 5'-phosphoramidite of guanosine 6. To a solution of N^2-(4-t-butyl)-benzoyl-2',3'-di-O-acetylguanosine (1.56 g, 3 mmol) in dichloromethane (20 ml) was added N,N-diisopropylmethylphosphonamidic chloride (1.2 ml, 6 mmol) followed by N,N-diisopropylethylamine (2 ml, 12 mmol) and the reaction stirred for 60 min. The reaction mixture was then diluted with ethylacetate (60 ml) and poured into saturated sodium chloride solution (40 ml) and extracted. The ethylacetate layer was washed with saturated sodium chloride solution (3 x 40 ml), dried over magnesium sulfate, filtered and evaporated with dry toluene. The residue was dissolved in dry toluene (20 ml) and precipitated from chilled hexane (200 ml) filtered and dried to give 6 (1.85 g, 90%). ^{1}H-NMR ($CDCl_3$): 8.0 (s, 1H) H-8; 7.94 (d, 2H) arom; 7.49 (d, 2H) arom; 6.04 (m, 2H) H-1' and H-2'; 5.85 (m, 1H) H-3'; 4.34 (m, 1H) H-4'; 3.73 (m, 2H) H-5'; 2.16 (s, 3H) acetate; 2.07 (s, 3H) acetate; 1.1 (s, 9H) t-butyl. ^{31}P-NMR ($CDCl_3$): +150.5 and +149.3.

Synthesis of partially protected lariat (7) and its deprotections to give either lariat (1) or (2). To a solution of (4) (250 mg, 0.2 mmol) and (6) (690 mg, 1 mmol) in dry acetonitrile (10 ml) was added tetrazole (690 mg, 10 mmol) and the reaction stirred for 1 h at 20 °C. The reaction mixture was then oxidized with iodine in tetrahydrofuran:pyridine:water (8:1:1 v/v/v) and stirred 10 min at 20 °C. The reaction mixture was then poured into 5% sodium bisulphite (100 ml) and extracted with dichloromethane (4 x 100 ml). The combined extracts were evaporated, and the residue was partially purified by silica gel chromatography. The column was washed with 4% ethanol-dichloromethane mixture (100 ml) and then the product was washed off with 20% methanol-dichloromethane mixture. The solvent was removed and then the residue was identified as to be 7. ^{31}P-NMR ($CDCl_3$): -0.8, -1.34, -1.44 and -1.88 (sole signals in ^{31}P-NMR spectra from 3'→ 5'

phosphodiester and 2'→5' phosphotriester of 7). It was divided into two halves and deprotected as follows:

(a) The first half of the partially protected trimer 7 was dissolved in dioxane (6 ml) and syn-4-nitrobenzaldoxime (0.16 g, 1 mmol) and tetramethylguanidine (0.12 g, 1 mmol) were added then water (6 ml). The reaction was stirred for 48 h and then aqueous ammonia (d=0.88) was added and the reaction stirred for 4 days. The volatile matters were then removed, and the residue dissolved in water (20 ml) and washed with dichloromethane (6 x 40 ml). The aqueous layer was evaporated, the dissolved in water and purified by DEAE Sephadex chromatography (15 cm x 1 cm) in 1.200 od_{260} batches using the following linear gradients: (1) 0.001 M TEAB (400 ml, pH 7.2) to 0.15 M TEAB (400 ml, pH 7.2); (2) 0.15 M TEAB (500 ml, pH 7.2) to 0.25 M TEAB (500 ml, pH 7.2). The elution profile is shown in Fig. 1. The product 1 was eluted at 0.21 M TEAB. The desired fractions were collected, evaporated and checked by Hplc (R_t=21.7 min) (panel A in Fig. 3) and ^1H NMR (Figs. 4, 6 and 8). Yield: 36.5% (730 $o.d._{260}$). ^{31}P -NMR (D_2O, 8.5% aq. H_3PO_4 as ext. standard at 30 °C): -2.0 (3'→5' phsphate) and -2.8 (2'→5' phosphate) (Fig. 12).

(b) The second half of the trimer 7 was dissolved in a small volume of dry tetrahydrofuran and then treated with liquid ammonia for 48 h at 20 °C. The liquid ammonia was then allowed to evaporate and the residue was dissolved in aqueous ammonia (d=0.88) and stirred for 4 days at 20 °C. The volatile matters were then removed, and the residue dissolved in water (20 ml) and washed with dichloromethane (6 x 40 ml). The aqueous layer was then evaporated, and dissolved in water and purified by DEAE Sephadex A25 chromatography (15 cm x 1 cm) in batches of 1.200 $o.d._{260}$. The following linear gradients were used: (1) 0.001 M triethylammonium bicarbonate (TEAB) (400 ml, pH 7.2) to 0.1 M TEAB (400 ml, pH 7.2); (2) 0.1 M TEAB (500 ml, pH 7.2) to 0.17 M TEAB (500 ml, pH 7.2). The elution profile is shown in Fig. 2. The product 2 was eluted at 0.15-0.16 M TEAB. The desired fractions were collected, evaporated and checked by Hplc (R_t=20.1 min) (panel B in Fig. 3) and ^1H NMR (Figs. 5, 7 and 9). Yield: 52% (1.040 $o.d._{260}$). ^{31}P-NMR (D_2O, 8.5% aq. H_3PO_4 as ext. standard at 30 °C): -1.9 (3'→5' phosphate) and -2.6 (2'→5' phosphate) (Fig. 13).

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References

1. Wallace, J.C. and Edmonds, M. Prod. Natl. Acad. Sci. U.S.A., 80, 950 (1983).
2. a) Weissmann, C. Nature, 311, 103 (1984).
   b) Keller, W. Cell, 39, 432 (1984).

3. Ruskin, B., Krainer, A.R., Matiatis, T. and Green, M.R. Cell, 38, 317 (1984).
4. Padgett, R.A., Konarska, M.M., Grabowski, P.J., Hardy, S. and Sharp, P.A. Science, 225, 898 (1984).
5. Hata, T., Sekine, M., J.A.C.S., 107, 5813 (1986).
6. Damha, M.J., Pon, R.T., Ogilvie, K.K. Tetrahedron Lett., 26, 4839 (1986).
7. Pathak, T. and Chattopadhyaya, J.B. Acta Chemica Scand. B39, 799 (1985).
8. Reese, C.B. and Skone, P.A. Nucleic Acids Res., 5215 (1985).
9. Kwiatkowski, M. and Chattopadhyaya, J. Chemica Scripta 20, 193 (1982).
10. Reese, C.B., Titmus, R.C. and Yau, L. Tetrahedron Lett. 2727 (1978).
11. For a review: Reese, C.B. Tetrahedron 34, 3143 (1978).
12. Jones, S.S., Rayner, B., Reese, C.B., Ubasawa, A. and Ubasawa, M. Tetrahedron 36 3075 (1980).
13. Kwiatkowski, M., Heikkilä, J., Björkman, S., Seliger, H. and Chattopadhyaya, J. Chem. Scr. 22, 30 (1983).
14. Sandström, A., Kwiatkowski, M. and Chattopadhyaya, J. Acta Chem. Scand. B39, 273 (1985).
15. Zhou, X-X., Sandström, A. and Chattopadhyaya, J. Chem. Scr. 26, 241 (1986).
16. Jones, S.S., Reese, C.B., Sibanda, S. and Ubasawa, A. Tetrahedron Lett. 22, 4755 (1981).
17. Nyilas, A. and Chattopadhyaya, J. Acta Chem. Scand. Ser. B (in press).
18. Zhou, X-X. and Chattopadhyaya, J. Tetrahedron (in press).
19. Kumar, G., Celewicz, L. and Chladek, S. J. Org. Chem. 634 (1982).
20. Beancage, S.L. and Caruthers, M.H. Tetrahedron Letts. 1859 (1981).
21. Ogilvie, K.K., Nemer, M.J. and Gillen, M.F. Tetrahedron Letts. 1669 (1984).
22. Kierkzek, R., Kopp, D.W., Edmonds, M. and Caruthers, M.H. Nucl. Acid Res., 14, 4751 (1986).
23. Kwiatkowski, M., Sandtröm, A., Balgobin, N. and Chattopadhyaya, J. Acta Chem. Scand. B38 721 (1984).
24. Chattopadhyaya, J.B. and Reese, C.B. J.C.S. Chem. Comm. 639 (1978).
25. Jones, S.S. and Reese, C.B. J.C.S. Perkin I. 2762 (1979).
26. Davis, D.G. and Bax, A. J. Am. Chem. Soc. 107, 7197 (1985).