The Cordycepin Analogue of 2,5A and Its *Threo* Isomer. Chemical Synthesis, Conformation and Biological Activity

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A new synthesis of the cordycepin analogue of 2,5A and its *threo* isomer is reported along with an assessment of their conformations by circular dichroism spectroscopy. Evidence is also presented showing that these compounds are stable against 2,5A-specific phosphodiesterase and are not able to activate the 2,5A-dependent endoribonuclease, possibly due to a reduced binding to the latter enzyme as compared to that of 2,5A.

The antiviral and antitumor activity of interferon in several cell-virus systems have been attributed to the interferon-induced enzyme, 2,5A synthetase, and its 2,5A product (*1*) which activates an endoribonuclease. This 2,5A-activated endoribonuclease then cleaves messenger and ribosomal RNAs, thus acting as a potent inhibitor of protein synthesis both in cell-free systems and in intact cells. The administration of interferon also triggers a 4- to 6-fold increase of a specific 2′-phosphodiesterase (2′PDi) which degrades the (2′→5′)phosphodiester bond of the 2,5A and thus destroys the antiviral activity of interferon. Recent reviews have discussed these interferon-induced mechanisms.[1-4]. Several synthetic analogues of 2,5A have been made in the last few years in order to explore the biological role of the 2,5A system and also to develop effective chemotherapeutic agents that are capable of acting as an interferon substitute.[5-12] Doetsch *et al.*[13] first reported the preparation and the biological evaluation of a cordycepin analogue (*2*) of 2,5A. They found that this cordycepin analogue was a more potent inhibitor of protein synthesis than was 2,5A (*1*) in lysed rabbit reticulocytes and that *2* also could prevent transformation of human lymphocytes infected by Epstein-Barr virus.[6] Suhadolnik *et al.*[18] also reported that the cordycepin analogue bound to and activated the 2,5A-dependent endoribonuclease. On the other hand, Sawai *et al.*[10] showed that *2* was devoid of activity against translation in rabbit reticulocyte lysates and L cell extracts; however, it was able to bind to, but unable to activate, the 2,5A-dependent endoribonuclease. No independent confirmation of either of these studies has yet been reported. We herein report our studies on a new synthesis of the cordycepin analogue of 2,5A and its *threo* isomer (*3*), their conformations assessed by their stacking ⇌ destacking properties using circular dichroism (CD) spectroscopy and, subsequently, present evidence regarding their biological role in the 2,5A system.

Chemistry

Charubala and Pfleiderer[14] first prepared the cordycepin trimer core, (*4*), in 13 steps starting from cordycepin, but did not convert it to its triphos-

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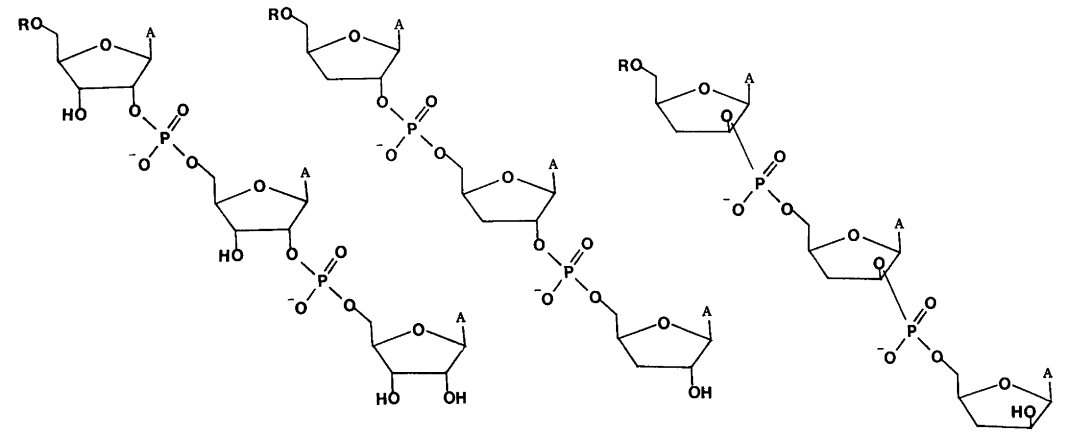
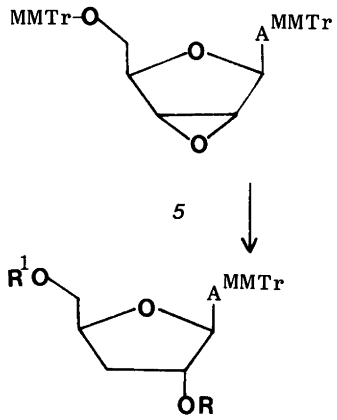
phate (*2*) which is a prerequisite for the biological activity. Sawai *et al.*[10] subsequently converted *4* into *2* using T_4 polynucleotide kinase. In our new 10-step procedure, readily accessible and appropriately protected *ribo* and *lyxo* epoxides, *5* and *6*, were employed as starting materials for the synthesis of the triphosphates *2* and *3*. Lithium aluminum hydride-promoted ring-opening reactions[20] of *5* and *6* gave *7* and *8* in 88 and 62 % yields respectively. Both the exocyclic amino and the 5′-hydroxyl functions of the building blocks, *9*, *10*, *16* and *17*, were protected by the same 4-methoxytriphenylmethyl (MMTr) group since one can remove the 5′-MMTr group selectively (*vide infra*) over the N^6-MMTr group from these building blocks allowing the further chain extension reaction to take place at the 5′ end.

The acetylation of the 3′-hydroxyl group of *7* and *8* gave quantitatively *9* and *10* which were not isolated; they were directly subjected to selective detritylation by treatment with a saturated solution of zinc bromide in nitromethane at 20 °C, to give *11* and *12* in 61 and 74 % yields respectively. The 2-chlorophenylphosphates (*13*) and (*14*) were then prepared in almost quantitative yields by reacting *7* and *8* with 2-chlorophenylphosphoro-bis-(1,2,4-triazolide) (*15*) using our conditions.[21] A slight excess of *13* and *14* were reacted with *11* and *12* respectively using 2-mesitylenesulfonyl chloride and 1-methylimidazole[22] in dry pyridine at 20 °C to give the fully protected dinucleotides *16* and *17*, in 91 and 94 % yields, respectively, after column chromatographic purification. The 5′-hydroxyl functions of *16* and *17* were then selectively unmasked, leaving the N^6-MMTr groups intact, to give *18* and *19*, in 85 and 75 % yields respectively, using the zinc bromide-promoted selective removal of the 5′-MMTr group. Fully protected trinucleotides, *20* and *21*, were finally obtained in 91 and 96 % yields by reacting *13* and *14* respectively, with the appropriate phosphodiester blocks, under usual conditions. Compounds *20* and *21* were fully deprotected by the following sequential treatments: (1) *syn*-4-nitrobenzaldoximate ion[23] for 18 h at 20 °C; (2) aqueous ammonia for 24 h; (3) 80 % acetic acid for 12 h at 20 °C. The deprotected cores were then purified on DEAE Sephadex A-25 columns using a linear gradient of triethylammonium hydrogen carbonate buffer, as detailed in the experimental section, to give *4* and *4a* in 78 an 83 % yields. Compounds *4* and *4a* were subsequently converted to their triphosphates *2* and *3* in 42 and 70 % yields respectively using phosphorous oxychloride in trimethylphosphate for selective monophosphorylation[24] of the 5′-hydroxyl function followed by a condensation step with bis-(tributylammonium)pyrophosphate at 20 °C (see experimental section for details).





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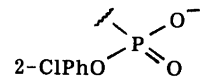
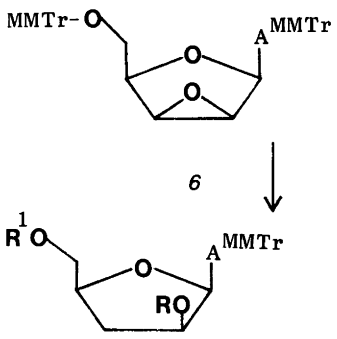
9; R = Ac; R^1 = MMTr

11; R = Ac; R^1 = H

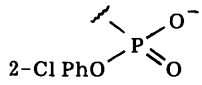
13; R =  ; R^1 = MMTr



8; R = H; R^1 = MMTr

10; R = Ac; R^1 = MMTr

12; R = Ac; R^1 = H

14; R =  ; R^1 = MMTr

A^{MMTr} = 6-*N*-(4-methoxytriphenylmethyl)-9-adeninyl-.

Optical properties of cordycepin analogues *2* and *3*

The UV and CD spectra (Figs. 1–4) of the two trinucleotides, *2* and *3*, are superficially very similar although the hyperchromicity and the magnitude of the low energy CD couplet about 260 nm are both lower in the case of *3*. These observations are somewhat misleading as the variable temperature UV and CD spectra both indicate that, in fact, the "stacking ability" of the two trimers is very similar: they are both good "stackers". Fitting the data to a van't Hoff plot gave in both cases a $H_{stacking}$ ~5.0±0.3 kcal mol^{-1}. Although *2* and *3* are stacked to similar extent, the geometries of their ordered states are different. The magnitude of the exciton coupling associated with the low energy 'B_{2u}' transition (260 nm) was lower for stacked *3* than for *2*. However, the optical activity associated with the 'E_{1u}' transition about 218 nm was enhanced for *3* with respect to *2*. This was evidenced by the relative magnitudes of the 270 nm and 218 nm CD bands.

Comparison of the present data with that published by us previously[25] is very revealing. The relative stacking ability of *2* and *3* is very similar to that of Ar2'p5'Ar2'p5'Aa and Aa2'p5'Ar2'p5'Aa where again, in the latter, the apparently reduced lower energy CD is offset by a higher 218 nm CD.[25] This variable CD intensity relationship is quite apparent when inspecting our earlier data. Of great importance here is the direct comparison of the spectra of *2* and *3* with the 2,5A (*1*).[25] Although both synthetic analogues are seemingly more stacked than the natural trimer, there are differences in the stacked state geometry. The ratio of the CD of *2* to that of natural 2,5A (*1*) is constant over all measured wavelengths, indicating that the relative orientations of the electronic transitions in the purine bases are the same. This is not true for *3*. The conclusions that are drawn from these considerations are, therefore, quite clear. The stereochemistry of the ordered state of *2* is sufficiently like that of the 2,5A that it can very closely compete with *1* in its biochemical properties.

Biological activity

Stability of cordycepin analogues against 2'→5'-specific phosphoriesterase (2'PDi). The 2,5A analogues of two cordycepin trimer cores, with

erythro and *threo* configurations, (*4*) and (*4a*) respectively, were found to be stable against partly purified 2'PDi. After 1 h of incubation at 30 °C, no detectable degradation (<1 %) was observed, while under a similar condition, 84 % of 2,5A core (*1a*) was hydrolysed to its dimer. In order to investigate if the cordycepin analogues were able to bind to 2'PDi without being hydrolysed, each cordycepin analogue, (*4*) or (*4a*), was mixed with *1a* in equimolar amounts in a 2'PDi assay. The presence of (*4*) or (*4a*) did not result in any decrease in the rate of degradation of the 2,5A core implying that the analogues (*4*) and (*4a*) bind to 2'PDi poorly.

Binding to and activation of 2,5A-dependent endoribonuclease (RNase L). In binding assays, the postmitochondrial fractions (S_{10} lysates) of L_{929} and EAT cells were incubated with ppp(A2'p)$_3$A-^{32}P-pCp with several dilutions of the competing compounds, (*1*), (*2*) or (*3*). The efficiency of a particular analogue to displace the ^{32}P probe from the binding protein (presumptive RNase L) is a measure of its affinity for that protein. It emerged from these studies that, while 2,5A (*1*) displaced 50 % of the ^{32}P probe at 0.5 nM concentration, the cordycepin analogue (*2*) required a concentration 50-fold higher, 25 n*M*, and the *threo* analogue (*3*) required more than 1000-fold excess, 600 nM, for the same ^{32}P probe displacement (Table 1). The same S_{10} lysates (L_{929} and EAT) were also used in the assay for the activation of RNase L. When 2,5A at 3 nM was incubated with S_{10} lysate, a characteristic and reproducible pattern of ribosomal RNA cleavage was generated as shown in Figure 5. No such pattern could be seen without the addition of 2,5A. The two analogues, (*2*) and (*3*), were then tested in order to determine if they activated the RNase L. When the assay contained L_{929} lysate, the specific cleavage pattern, as observed with 3 nM of 2,5A, was seen also with 33 nM of corcycepin analogue (*2*), while the *threo* cordycepin analogue (*3*) failed to show a similar activation of RNase L at up to 3 μM concentration (Figure 5). Activation studies using EAT extract showed no cleavage for the cordycepin analogue (*2*) at 33 nM and again no activation was observed by the *threo* cordycepin analogue (*3*) at 330 nM; 2,5A at 3 nM resulted in cleavage of rRNA (Figure 5). In another experiment, both analogues (*2*) and (*3*) were tested at 500 μM concentration for their ability to activate the RNase L in EAT extracts but no rRNA cleavage could be seen (not shown).

Discussion

Contradictory results have been reported concerning the biological role of the cordycepin analogue (*2*) regarding its ability to activate RNase L.[10,13,18,19] The 3'-hydroxyls of the triphosphorylated trimer have been considered critical, not in binding to, but for activation of the 2,5A-dependent nuclease RNase L.[10,19] Using longer incubation times, Eppstein *et al.*[19] found a slight inhibition of protein synthesis due to the ability of the cordycepin analogue (*2*) to activate RNase L. This activation was evident using the L_{929} extract

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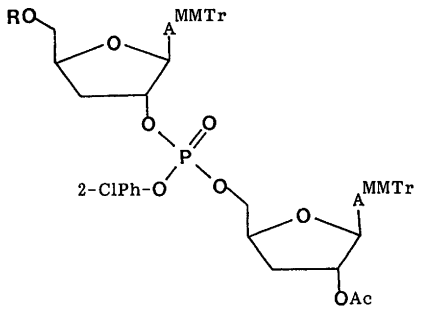
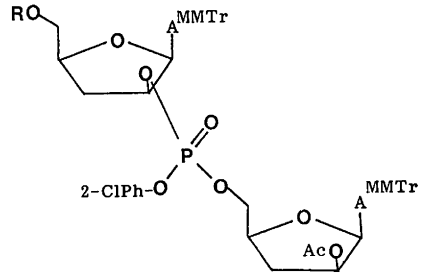


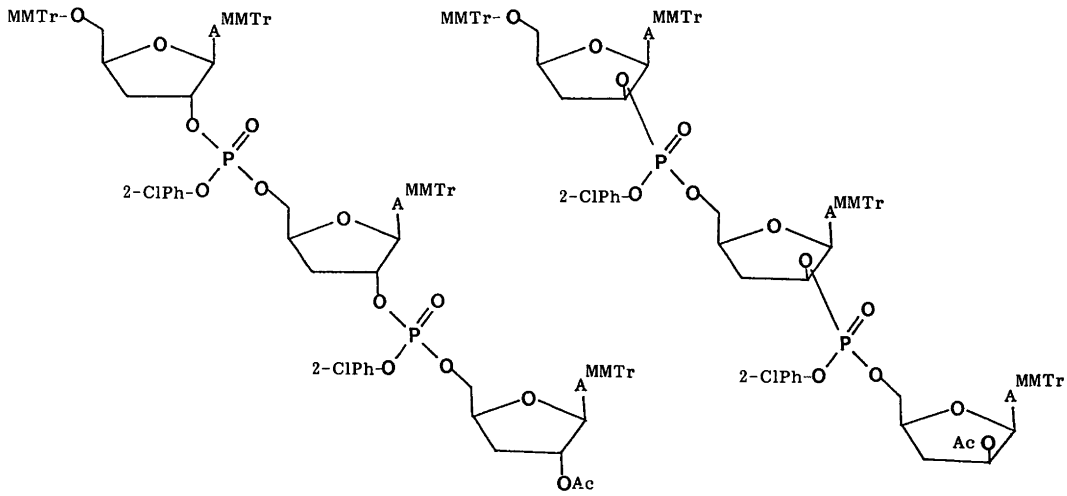
16; R = MMTr

18; R = H



17; R = MMTr

19; R = H



20 *21*

but not the EAT extract. The reports of Sawai *et al.*[10] and Eppstein *et al.*[19] that the cordycepin analogue (*2*) does not inhibit protein synthesis in rabbit reticulocyte lysate contradict others[13,18] which describe the cordycepin tetramer as even better than 2,5A (*1*) inhibiting the protein synthesis in rabbit reticulocyte lysates through activation of the RNase L. It has emerged through the present work that both *erythro* and *threo* analogues of 3'-deoxyadenosine can bind to L_{929} and EAT lysates, but their bindings were 50 and 1000 times less effective, respectively, than that of 2,5A. The present result of 50-fold lower binding of *2* compared to that of 2,5A is even less than that reported by Sawai *et al.*[10] who found 2- to 10-fold lower binding. This reduced binding of *2* supports the data that it is at least 10-fold less potent than 2,5A in activating the L_{929} endonuclease. It is possible that the inability of the cordycepin analogues to activate EAT endonuclease could be due to the presence of an inhibitor in that particular lysate. The difference in effect of the two cordycepin analogues was not due to differences in their stabilities since their cores, (*4*) and (*4a*), were both stable against the 2'PDi. The lack of activation of RNase L found in this investigation corresponds to the decrease in binding of *2* to RNase L and confirms the results of Eppstein *et al.*[19] that *2* is at least 10- to 100-fold less potent than 2,5A in activating RNase L.

Table 1. Binding of ppp(A2'p)$_2$A (*1*) and cordycepin analogues (*2*) and (*3*) to RNase L in L_{929} and EAT extracts.

Compound	Concentration / nM for 50 % displacement of ppp(A2'p)$_3$A ^{32}P pCp	
	L_{929}	EAT
1	0.5	0.5
2	25	70
3	600	2000

The biochemical activity of 2,5A (*1*) probably depends on its precise stereochemical structure. Our present CD study revealed that it is *2* which mimics the stereochemical fit of *1* more closely than that of *3* and this corresponds to the biochemical activities found.

Experimental

^{1}H NMR spectra were measured at 90 MHz with a Jeol FX 90Q spectrometer and Jeol GX 270 spectrometer at 270 MHz using tetramethylsilane as internal standard in the δ scale. ^{31}P NMR spectra were recorded at 36 MHz in the same solvent mixture as for ^{1}H NMR using 85 % phosphoric acid as an external standard in the δ scale. UV

absorption spectra were recorded with a Varian-Cary 2200 spectrophotometer. Reactions were monitored by using PEI cellulose F (A) and Merck precoated silica gel 60 F_{254} plates (B,C,D,E) using the following solvent systems: (A) 0.5 M sodium chloride; (B) propanol/ammonia/water (11:7:2, v/v/v); (C) isopropyl alcohol/ammonia/water (11:2:7, v/v/v); (D) isobutyric acid/ammonia/water (66:11:33, v/v/v); (E) 1 M sodium acetate/ethanol (2:8, v/v).

High performance liquid chromatography (HPLC) was performed on a RP 18 column using gradient A: 0.0075 M tetrabutylammonium sodium sulfate in water, and gradient B: 0.0075 M tetrabutylammonium sodium sulfate in acetonitrile/water (30:70, v/v) at 20 °C.

The 2′→5′-specific phosphodiesterase used in the stability studies was partly purified from EAT cells grown intraperitoneally in mice.[15] The assay conditions were the same as described earlier[15] and the concentration of trimers was 30 μm. Degradation was determined by HPLC.[15] S_{10} extracts from L_{929} and EAT cells used for radiobinding and ribosomal RNA cleavage assays were prepared as already described.[16] The radiobinding assay and ribosomal RNA cleavage through activation of the endonuclease RNase L were performed according to literature procedures.[7,17] The 2,5A was purchased from P.L. Biochemicals, Inc., Stockholm.

6-N-(4-methoxytriphenylmethyl)-5′-0-(4-methoxytriphenylmethyl)-2′-triethylammonium-(2-chlorophenyl)phosphoro-3′-deoxyadenosine (13). Compound 7 (1 g, 1.25 mmol) was coevaporated with dry pyridine twice and then taken up in dry pyridine (20 ml). A 0.25 M solution of *o*-chlorophenylphosphoro-bis-(1,2,4-triazolide) (*15*) in acetonitrile (10 ml) was added. The reaction mixture was stirred for 30 min at 20 °C and then quenched with 0.5 M triethylammonium bicarbonate solution (10 ml). It was poured into saturated sodium hydrogen carbonate solution (50 ml) and extracted with dichloromethane (2×50 ml). The organic layers were combined and extracted with water (2×100 ml). The dichloromethane extract was evaporated and coevaporated with toluene. The residue was dissolved in dichloromethane (10 ml) and precipitated from hexane (60 ml) giving a white powder (1.19 g, 86 %). ^{1}H NMR ($CDCl_3$): 7.85 (s, 2H) H-8, H-2; 7.5–6.63 (m, 32H) arom; 6.16 (brs, 1H) H-1′; 5.32 (m, 1H) H-2′; 4.53 (m, 1H) H-4′; 3.7 (*s*, 6H) MMTr; 3.26–2.68 (m, 8H) H-5′ and CH_2 of triethylammonium; 2.23 (m, 2H) H-3′; 1.13 (t, 9H) CH_3 of triethylammonium.^{31}P NMR ($CDCl_3$): −6.8.

6-N-(4-methoxytriphenylmethyl)-2′-0-acetyl-3′-deoxyadenosine (11). Compound 7 (0.5 g, 0.63 mmol) was coevaporated with dry pyridine twice then redissolved in dry pyridine (10 ml). Dry acetic anhydride (0.18 ml, 1.89 mmol) was then added. The reaction was complete after 2 h; the reaction mixture was poured into saturated sodium hydrogen carbonate solution (10 ml) and extracted with dichloromethane (3×10 ml). The organic layer was evaporated and coevaporated with toluene. This residue, *9*, was directly deprotected with saturated zinc bromide solution (50 ml) in nitromethane at 20 °C. The reaction was

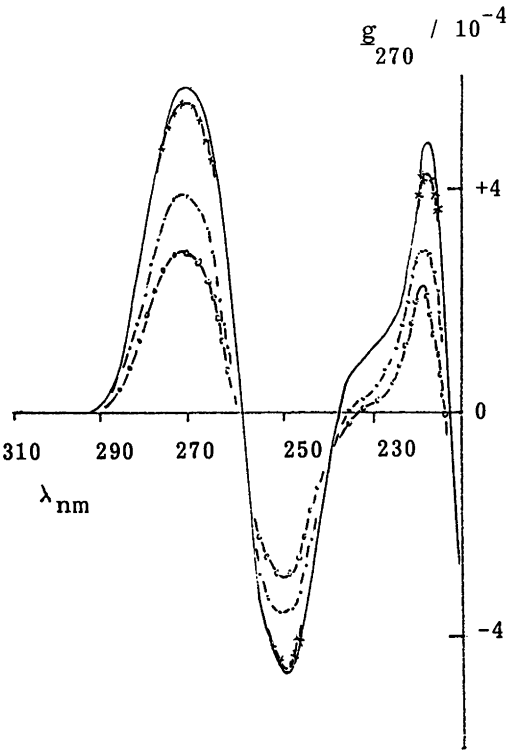


Fig. 1. The CD spectrum of *2* at pH 7.4 (0.01 M phosphate buffer). ——— 4 °C, —×— 17 °C, —|— 60 °C, —○— 89 °C.

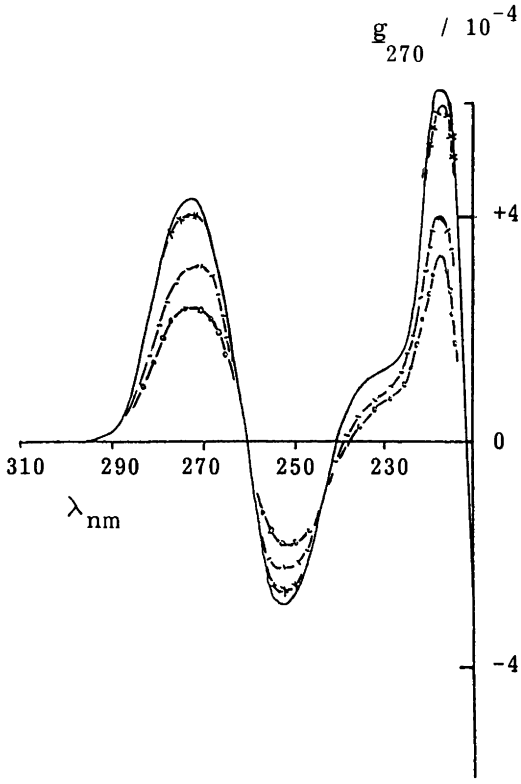


Fig. 2. The CD spectrum at *3* at pH 7.4 (0.01 M phosphate buffer). ——— 4 °C, —×— 17 °C, —|— 60 °C, —○—89 °C.

complete within 1 min (appearance of orange colour) and it was quanched with aqueous ammonia. Volatile matter was removed *in vacuo*; the residue was taken up in dichloromethane (10 ml) and extracted with saturated sodium hydrogen carbonate (2×10 ml). The organic layer was evaporated. The residue was subsequently purified by short column silica gel chromatography. Appropriate fractions were collected and evaporated giving a glass which was precipitated from hexane to give *11* (0.22 g, 65 %).

Compound 9: ^{1}H NMR ($CDCl_3$) 8.02 (*s*, 1H) H-8; 7.92 (s, 1H) H-2; 7.5–6.63 (m, 28H) arom.; 6.11 (d, J = 1.1 Hz, 1H) H-1′; 5.70 (m, 1H) H-2′; 4.50 (m, 1H) H-4′; 3.78 (s, 6H) MMTr; 3.43 (m, 2H) H-5′; 2.64–2.11 (m, 5H) H-3′ and acetate.

Compound 11: ^{1}H NMR ($CDCl_3$) 8.02 (s, 1H) H-8; 7.85 (s, 1H) H-2; 7.5–6.71 (m, 14H) arom.; 5.90 (*d*, J = 3.5 Hz, 1H) H-1′; 5.57 (m, 1H) H-2′; 4.54 (m, 1H) H-4′; 4.2–3.48 (m, 2H) H-5′; 3.8 (s, 3H) MMTr; 2.97 (m, 2H) H-3′; 2.11 (s, 3H) acetate.

6-N-(4-methoxytriphenylmethyl)-5′-0-(4-methoxytriphenylmethyl)-3′-deoxyadenylyl-(2′→5′)-6-N-(4-methoxytriphenylmethyl-2′-0-acetyl-3′-deoxyadenosine: (16) *and 6-N-(4-methoxytriphenylmethyl)-3′-deoxyadenylyl-(2′→5′)-6-N-(4-methoxytriphenylmethyl)-2′-0-acetyl-3′-deoxyadenosine* (18). Compound *13* (0.353 g, 031 mmol) and *11* (0.147 g, 0.258 mmol) were coevaporated together with dry pyridine (1 ml) and redissolved in dry pyridine (1 ml). A 12 mmol solution of 1-methylimidazole in dry pyridine (1.3 ml) and 2-mesitylenesulfonyl chloride (0.169 g, 0.77 mmol) were added. The reaction mixture was stirred for 30 min at 20 °C and then quenched with water. The volatile matter was removed *in vacuo* to give a glass of *16* (91 %). A saturated solution of zinc bromide (6 ml) in nitromethane was then added to the residue with vigorous stirring. The reaction was quenched by addition of water. The volatile matter was removed *in vacuo* giving a foam which was fractionated by short column chromatography on silica gel. Appropriate fractions were collected and concentrated *in vacuo* giving *18* as a glass which was precipitated from hexane (0.165 g, 85 %).

Compound 16: ^{1}H NMR ($CDCl_3$) 8.06, 8.01, 7.97, 7.94, 7.92, 7.89, 7.78 (each s, 4H) H-2 and H-8; 7.45–6.65 (m, 46H) arom.; 6.17 (d, J = 10.6 Hz, 1H) and 5.93 (d, J = 8.3 Hz, 1H) H-1′ and H-1″; 5.65 (m, 2H) H-2′ and H-2″; 4.75–4.35 (m, 4H) H-4′, H-4″ and H-5″; 3.75 (s, 9H) MMTr; 3.35 (m, 2H) H-5′; 2.9–2.1 (m, 4H) H-3′ and H-3″; 2.07, 2.06 (two s, 6H) acetates. ^{31}P NMR: −7.7 and −8.1.

Compound 18: ^{1}H NMR ($CDCl_3$) 8.00, 7.96, 7.95, 7.93, 7.89, 7.8 (each s, 4H) H-2 and H-8; 7.6–6.65 (m, 32H) arom.; 6.00 (*s*, 1H) and 5.90 (two d, J = 4.2 Hz, J = 4.8 Hz, 1H) H-1′ and H-1″; 5.6 (m, 2H) H-2′ and H-2″; 4.65–4.2 (m, 4H) H-4′ and H-4″ and H-5″; 3.77, 3.76, 3.75 (each s, 6H) MMTr; 3.65 (m, 2H) H-5′; 2.9–2.15 (m, 4H) H-3′ and H-3″; 2.1, 2.09 (two s, 6H) acetates. ^{31}P NMR: −7.7, −8.3.

6-N-(4-methoxytriphenylmethyl)-5′-0-(4-methoxytriphenylmethyl)-3′-deoxyadenylyl-(2′→5′)-6-N-(4-methoxytriphenylmethyl-3′-deoxyadenylyl-

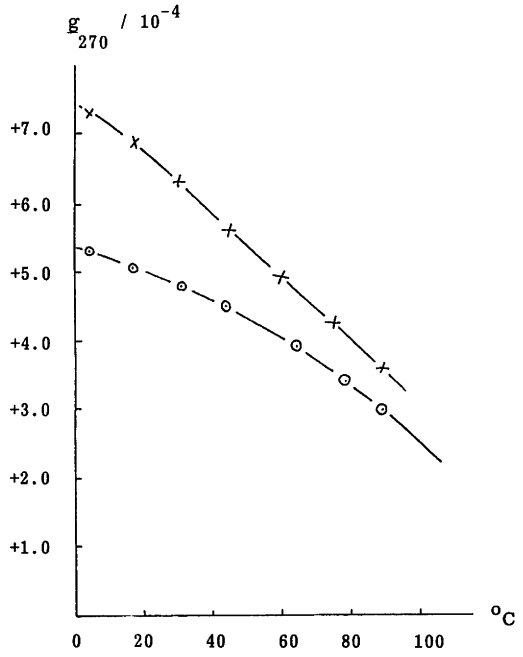


Fig. 3. Plot of dissymetry *g* factor as a function of temperature at 270 nm in 0.01 M phosphate buffer at pH 7.4. —×— *2*, —⊙— 3.

*(2′→5′)-6-*N*-(4-methoxytriphenylmethyl)-2′-*0*-acetyl-3′-deoxyadenosine:* (20) *and 3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenosine* (4). Compound *18* (99 mg, 0.078 mmol) and *13* (0.1 g, 0.085 mmol) were coevaporated with dry pyridine and redissolved in dry pyridine (1 ml). A 12 mmol stock solution of 1-methylimidazole in dry pyridine (0.5 ml) and 2-mesitylenesulfonyl chloride were then added. The reaction mixture was stirred for 30 min at 20 °C and quenched with water. Standard work-up and purification on a short silica gel column gave *20* (0.12 g, 76 %). Compound *20* was taken up in a dioxane/water mixture (1.2 ml, 1:0.2, v/v) and *syn*-4-nitrobenzaldoxime (0.019 g, 0.12 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (0.014 ml, 0.11 mmol) added and stirred for 24 h at 20 °C; then 7 M aqueous ammonia (5 ml) was added. After 1 day, the volatile matter was removed *in vacuo* and 80 % acetic acid solution was added and stirred for 12 h at 20 °C. Volatile matter was removed and the residue was partitioned between water (10 ml) and dichloromethane (10 ml). The aqueous phase was loaded on a DEAE Sephadex A-25 column using triethylammonium hydrogen carbonate (pH 7.6) for linear gradient elution (0.001 M–0.6 M). Compound *4* was thus obtained in 90 % yield (A_{260} units).

Compound 20: ^{1}H NMR ($CDCl_3$) 7.91, 7.85, 7.79, 7.71 (each s, 6H) H-2 and H-8; 7.6–6.5 (m, 64H) arom.; 6.05 (d, J = 11.7 Hz, 1H) and 5.92 (*d*, 13.5 Hz, 2H) H-1′, H-1″ and H-1‴; 5.59 (m, 3H) H-2′, H-2″ and H-2‴; 4.8–4.1 (m, 7H) H-4′, H-4″, H-4‴, H-5″, H-5‴; 3.68, 3.6 (each s, 12H) MMTr; 3.25 (m, 2H) H-5′; 2.7–2.0 (m, 6H) H-3′, H-3″, and H-3‴; 1.97, 1.92 (each s, 6H) CH_3 of acetyl. ^{31}P NMR: −7.6, −7.7, −8.0, −8.1.

Compound 4: ^{1}H NMR (D_2O) 8.09, 8.02, 7.99, 7.97, 7.85, 7.79 (each *s*, 6H) H-2 and H-8; 6.01 (s, 1H) 5.85 (s, 1H) 5.76 (d, J = 7 Hz, 1H) H-1′, H-1″ and H-1‴. ^{31}P NMR: −1.45; R_f (TLC): 0.65 (A); 0.6 (B); 0.8 (C); 0.55 (D); 0.2 (E). R_t (Hplc): 16.77 min. UV (water): λ max 253 nm (pH 2); 255 nm (pH 7); 255 nm (pH 12).

*6-*N*-(4-methoxytriphenylmethyl)-5′-*0*-(4-methoxytriphenylmethyl)-2′-triethylammonium-(2-chlorophenyl)phosphoro-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)adenine:* (14). Compound *8* (0.8

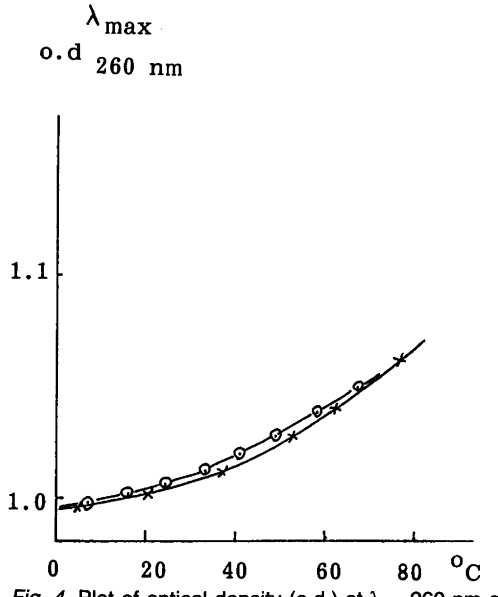


Fig. 4. Plot of optical density (o.d.) at λ_{max} 260 nm as a functioon of temperature (data normalized to o.d. =1.0 at 18 °C) in 0.01 M phosphate buffer at pH 7.4. —×— *2*, —⊙— 3.

g, 1 mmol) was coevaporated with dry pyridine twice, the residue was redissolved in dry pyridine (20 ml) and 0.25 M solution of *15* (8 ml) added and stirred for 30 min at 20 °C. The reaction was quenched by addition of 0.5 M triethylammonium hydrogen carbonate buffer (10 ml). Standard work-up and purification gave *14* (1.08 g, 98 %). ^{1}H NMR ($CDCl_3$): 8.16 (s, 1H) H-8; 7.96 (s, 1H) H-2; 7.3–6.71 (m, 32H) arom; 6.32 (d, 1H) H-1′; 5.16 (m, 1H) H-2′; 4.15 (m, 1H) H-4′; 3.78 (s, 6H) MMTr; 3.28 (m, 2H) H-5′; 2.99–2.52 (m, 8H) H-3′ and CH_2 of triethylammonium; 1.04 (t, 9H) CH_3 of triethylammonium. ^{31}P NMR: −6.7.

*6-*N*-(4-methoxytriphenylmethyl-5′-*0*-(4-methoxytriphenylmethyl)-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)-adenylyl-(2′→5′)-6-*N*-(4-methoxytriphenylmethyl)-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)-adenylyl-(2′→5′)-6-*N*-(4-methoxytriphenyl methyl)-2′-*0*-acetyl-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)adenine:* (21) *and 3′-deoxy-9-(β-D-threo-pentofuranosyl)-adenylyl-(2′→5′)-3′-deoxy-9-(β-D-threo-pentofuranosyl)-adenylyl-(2′→5′)-3′-deoxy-(β-D-pentofuranosyl)adenosine* (4a). Compound *19* (0.16 g, 0.126 mmol) and *14* (0.17 g, 0.152 mmol) were coevaporated together with dry pyridine and redissolved in dry pyridine (1 ml); a 12 mmol solution of 1-methylimidazole in dry pyridine (0.76 ml) and 2-mesitylenesulfonyl chloride (0.079 g, 0.38 mmol) were then added. The reaction mixture was stirred for 30 min at 20 °C then was quenched by adding water. The usual work-up gave a foam (0.27, 95 %) which was deprotected to give *4a* (92 mg, 70 %) using a procedure described for the preparation of *4*.

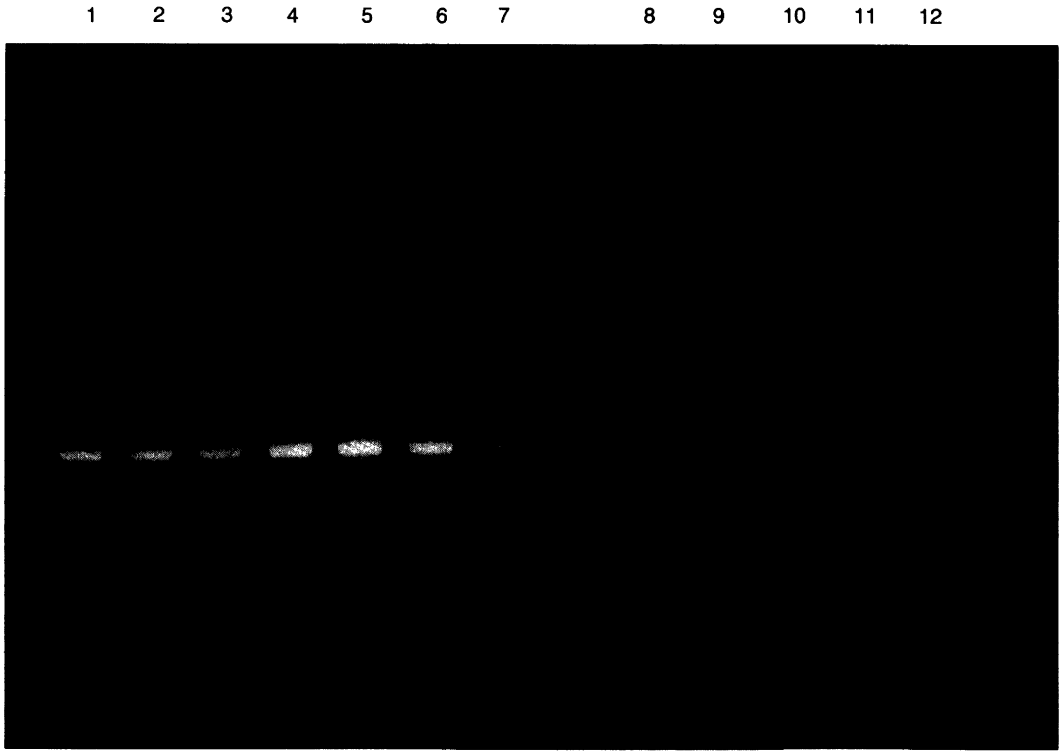


Fig. 5. Activation of the ppp2,5A-dependent RNase. Analysis of ribosomal RNA from L_{929} S10 lysates (lanes 1–7) and EAT S10 lysates (lanes 8–12). Assay conditions were as described in Materials and Methods. Lanes 1 and 8, 0 nM, lanes 2 and 9, 3 nM, lanes 3 and 10, 33 nM of *1*; lane 4, 3 nM, lanes 5 and 11, 33 nM of *2*; lanes 6 and 12, 330 nM of *3*; lane 7, 3 n*M* of *1* and 3 μM of *3*.

Compound 21: ^{1}H NMR ($CDCl_3$) 8.05, 8.04, 8.03, 8.02, 7.99, 7.95, 7.93, 7.91, 7.90, 7.89, 7.88, 7.85, 7.83, 7.82, 7.81, 7.80 (each s, 6H) H-2 and H-8; 7.5–6.48 (m, 64H) arom.; 6.25–6.07 (m, 6H) H-1′ and H-1″ and H-1‴; 5.6–5.12 (m, 3H) H-2′, H-2″ and H-2‴; 4.65–3.86 (m, 7H) H-4′, H-4″, H-4‴, H-5″, H-5‴; 3.68, 3.67, 3.66, 3.65, 3.62 (each s, 12H) MMTr; 3.43–3.22 (m, 2H) H-5′; 2.49–1.87 (m, 6H) H-3′, H-3″, H-3‴; 1.70, 1.68, 1.67 (each s, 6H) CH_3 of acetyl. 31 p) NMR: −7.25, −7.3, −7.3, −7.5, −7.7, −7.8.
Compound 4a: ^{1}H NMR (D_2O) 8.35, 8.16, 8.00, 7.71, 7.64 (each s, 6H) H-2 & H-8; 6.15 (s, 1H), 6.01 (d, J = 5.7 Hz, 1H), 5.93 (d, J = 5.7 Hz, 1H) H-1′, H-1″ and H-1‴;. ^{31}P NMR: −1.35. R_f (Tlc): 0.6 (A); 0.6 (B); 0.8 (C); 0.5 (D); 0.2 (E). R_t (Hplc): 16.30 min. UV (water): λ_{max} 253 pH (pH 2); 255 nm (pH 7); 255 nm (pH 12).

5′-0-triphospho-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenosine: (2). Dry *4* (10 mg, 0.0095 mmol) was suspended in trimethyl phosphate (0.5 ml) and chilled to 0 °C. Phosphorus oxychloride (0.032 ml, 1.27 mmol) was added with stirring for 3.5 h at 0 °C. Then a mixture of 0.5 M bis(tributylammonium)pyrophosphate in anhydrous *N,N*-dimethylformamide (2.5 ml) and tributylamine (0.25 ml) were added and stirred vigorously for 1 min. The reaction mixture was poured into 0.5 M trimethylammonium hydrogen carbonate solution (5 ml) and stirred for 3 h at 20 °C. The volatile matter was evaporated, the residue extracted with diethyl ether and loaded on a DEAE Sephadex A-25 column which was eluted with a linear gradient of 0.001–0.6 M triethylammonium hydrogen carbonate solution (pH 7.6). Appropriate fractions were collected, evaporated and coevaporated several times with water then lyophilised to give *2* (42 %, A_{260} units). R_f (Tlc): 0.0 (A); 0.1 (B); 0.6 (C); 0.15 (D). UV (water): λ_{max} 255 nm (pH 7). ^{31}P NMR (D_2O): −1.3, −1.5 (internucleotide phosphate); −10.5 (d, J = 5.3 Hz) (α-P); −23.25 (dd, J = 5.2 Hz, J = 5.3 Hz) (β-P); −10.9 (d, J = 5.3 Hz) (γ-P).

*6-N-(4-methoxytriphenylmethyl)-5′-0-(4-methoxytriphenylmethyl)-2′-0-acetyl-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)adenine:* (10) *and 6-N-(4-methoxytriphenylmethyl)-2′-0-acetyl-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)adenine:* (12). Compound *8* (0.6 g, 0.752 mmol) was coevaporated with dry pyridine twice and then redissolved in dry pyridine (5 ml). Dry acetic anhydride (0.21 ml, 2.26 mmol) was then added. The reaction was worked up using a procedure analogous to the preparation of *9* to give *10* (0.6 g, 95 %). Compound *10* was subsequently deprotected using a procedure similar to the preparation of *11* to give *12* (0.32 g, 78 %).
Compound 12: ^{1}H NMR ($CDCl_3$) 8.04 (s, 1H) H-8; 7.77 (s, 1H) H-2; 7.57–6.71 (m, 14H) arom; 6.31 (d, J = 6.0 Hz, 1H) H-1′; 5.55 (m, 1H) H-2′; 4.19 (m, 1H) H-4′; 4.1–3.62 (m, 2H) H-5′; 3.78 (s, 3H) MMTr; 2.58 (m, 2H) H-3′; 1.66 (s, 3H) acetate.

*6-N-(4-methoxytriphenylmethyl-5′-0-(4-methoxytriphenylmethyl)-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)adenylyl-(2′→5′)-6-N-(4-methoxytriphenylmethyl-2′-0-acetyl-3′-deoxy-9-(β-D-*threo*-pentofutanosyl)adenine:* (17) *and 6-N-(4-methoxytriphenylmethyl)-3′-deoxy-9-(β-D-*threo*-pentoduranosyl)-adenylyl-(2′→5′)-6-N-(4-methoxytriphenylmethyl)-2′-0-acetyl-3′-deoxy-9-(β-D-*threo*-pentofuranosyl)adenine* (19). Compound *14* (0.26 g, 0.24 mmol) and *12* (0.11 g, 0.2 mmol) were coevaporated with dry pyridine and redissolved in dry pyridine (1 ml); A 12 mmol stock solution of 1-methylimidazole in dry pyridine (1.2 ml) and 2-mesitylenesulfonyl chloride (0.13 g, 0.6 mmol) were then added. The reaction was worked up in the usual way giving *17* as a foam (0.2 g, 95 %). Compound *17* was deprotected and worked up in the usual way as described for the preparation of *11* to give *19* (0.23 g, 75 %).
Compound 17: ^{1}H NMR ($CDCl_3$) 8.07, 8.01, 7.99, 7.97 (each s, 4H) H-2 and H-8; 7.58–6.75 (m, 46H) arom; 6.33 (m, 2H) H-1′ and H-1″; 5.37–5.27 (m, 2H) H-2′ and H-2″; 4.26–3.98 (m, 4H) H-4′ and H-4″ and H-5″; 3.75 (s, 6H) MMTr; 3.43–3.3 (m, 2H) H-5′; 2.5–2.08 (m, 4H) H-3′ and H-3″; 1.77 (s, 6H) CH_3 of acetyl. ^{31}P NMR: −7.5, −7.9.
Compound 19: ^{1}H NMR ($CDCl_3$) 8.1, 8.03, 8.02, 7.98, 7.97, 7.93, 7.88 (each s, 4H) H-2 and H-8; 7.42–6.7 (m, 32H) arom.; 6.39 (d, J = 4.9 Hz, 1H), 6.30 (d, J = 4.9 Hz, 1H), 6.25 (d, J = 5.8 Hz, 1H), 6.18 (d, J = 5.8 Hz, 1H), H-1′ and H-1″; 5.50–5.24 (m, 2H) H-2′ and H-2″; 4.36–3.85 (m, 4H) H-4′ and H-4″ and H-5″; 4.0–3.45 (m, 2H) H-5′; 3.77, 3.76, 3.75 (each s, 6H) MMTr; 2.85–2.16 (m, 4H) H-3′ and H-3″; 1.83, 1.79 (each s, 6H) acetate. ^{31}P NMR: −7.5, −7.9.

*5'-O-triphospho-3'-deoxy-9-(β-D-*threo*-pentofuranosyl)adenylyl-(2'→5')-3'-deoxy-9-(β-D-*threo*-pentofuranosyl)-adenylyl-(2'→5')-3'-deoxy-9-(β-D-*threo*-pentofuranosyl)adenosine (3). It was prepared using essentially the same reaction condition as described for 2 (70 %) (A_{260} units). R_f (Tlc): 0.0 (A); 0.0 (B); 0.6 (C); 0.1 (D). UV (water): λ_{max} 255 nm (pH 7). ^{31}P NMR (D_2O): −1.3, −1.5 (internucleotide phosphate); (d, J = 10.3 Hz, 6.2 Hz) (α-P); −23.3 (dd, J = 5.2 Hz, J = 6.2 Hz) (β-P); −10.75 (d, J = 6.2 Hz) (γ-P).

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