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Polynucleotides. XXVI.[1] Synthesis of an AUG Analog, 8,2′-Anhydro-8-oxy-9-β-D-arabinofuranosyladenine Phosphoryl-(3′—5′)-uridylyl-(3′—5′)-guanosine

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A triplet AUG analog containing 8,2′-O-cycloadenosine in the first position was synthesized.

Starting from 2′-O-(2,4,6-triisopropylbenzenesulfonyl)-8-bromoadenosine (I), 5′-monomethoxytrityl-8,2′-O-cycloadenosine (IV) was synthesized *via* 8-oxy derivative (II) by the successive treatment with sodium acetate, monomethoxytrityl chloride and methanolic ammonia. Phosphorylation of compound IV with cyanoethyl phosphate and dicyclohexylcarbodiimide (DCC) at 18—20° for 5 days gave only 3′-cyanoethylphosphate (V) quantitatively. Treatment of V with methanolic ammonia gave 3′-phosphate (VI) in a relatively low yield with concomitant formation of 2′,3′-cyclic phosphate (VII).

Compound VI was then condensed with 2′-O-benzoyluridylyl-(3′—5′)-N^2,2′,3′-triisobutyrylguanosine (X) using DCC and after purification on a column of diethylaminoethyl (DEAE)-cellulose, 8,2′-O-cycloadenylyl-(3′—5′)-uridylyl-(3′—5′)-guanosine (XI) was obtained in a yield of 4.1%.

Compound XI showed hypochromicity of 7.3% at 257 nm and circular dichroism (CD) spectra taken at 0° and 20° revealed that this trinucleotide exists in a rather freely rotatable, unstacked form both at these temperatures.

In order to investigate the relation between conformation of the triplet adenylyl-(3′—5′)-uridylyl-(3′→5′)-guanosine (AUG), which is known to be recognized as the initiation or internal methionine codon in protein synthesis, and its biological functions we synthesized previously an AUG analog containing 8,5′-O-cycloadenosine.[3] As it is established[4] that the base moiety in 8,5′-cyclonucleoside is fixed at ϕ_{CN}[5] $\approx -40°$ and in 8,2′-cyclonucleoside it is fixed at $\phi_{CN} \approx -120°$, it might be of interest to synthesize an AUG analog having 8,2′-O-cycloadenosine (A°) at the first position.

For the synthesis of triplets, Khorana and coworkers[6] reported a general method starting from a dinucleoside monophosphate, which was then condensed with a 3′-monophosphate. Therefore, for the synthesis of A°pUpG, 8,2′-cyclonucleoside 3′-phosphate was planned to be synthesized as illustrated in Chart 1.

2′-O-(2,4,6-triispropylbenzeuesulfonyl)-8-Bromoadenosine[7] (I) was heated with sodium acetate in a mixture of acetic anhydride and acetic acid at 150° for 7 hr. After usual work up tetraacetate of I was obtained as a glass, which was treated with 2N NaOH at 0° for 5 min in 50% aqueous pyridine. By this procedure N^6-acetyl-2′-TPS-8-oxyadenosine (II) was obtained as a white powder in a over-all yield of 80%. From the ultraviolet (UV) absorption, $\lambda_{max}^{H_2O,H^+}$ 288.5 nm and $\lambda_{max}^{OH^-}$ 309.5 nm, the introduction of acetyl group to N^6 position was confirmed.

Compound II was then dissolved in pyridine and treated with monomethoxytrityl chloride to give 5′-trityl derivative (III) as a pale yellow oil. Compound III was then dissolved in

(1 Part XXV: M. Ikehara, S. Uesugi, and J. Yano, *J. Am. Chem. Soc.*, **96**, 4966 (1974).
2) Location: *6–1–1, Toneyama, Toyonaka, Osaka.*
3) M. Ikehara, T. Nagura, and E. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **22**, 123 (1974).
4) K. Tomita, T. Tanaka, M. Yoneda, T. Fujiwara, and M. Ikehara, *Acta Cryst.*, **A28**, S45 (1972).
5) J. Donohue and K.N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).
6) R. Lohrmann, D. Söll, H. Hayatsu, E. Ohtsuka, and H.G. Khorana, *J. Am. Chem. Soc.*, **88**, 819 (1966).
7) M. Ikehara and M. Kaneko, *Tetrahedron*, **26**, 4251 (1970).

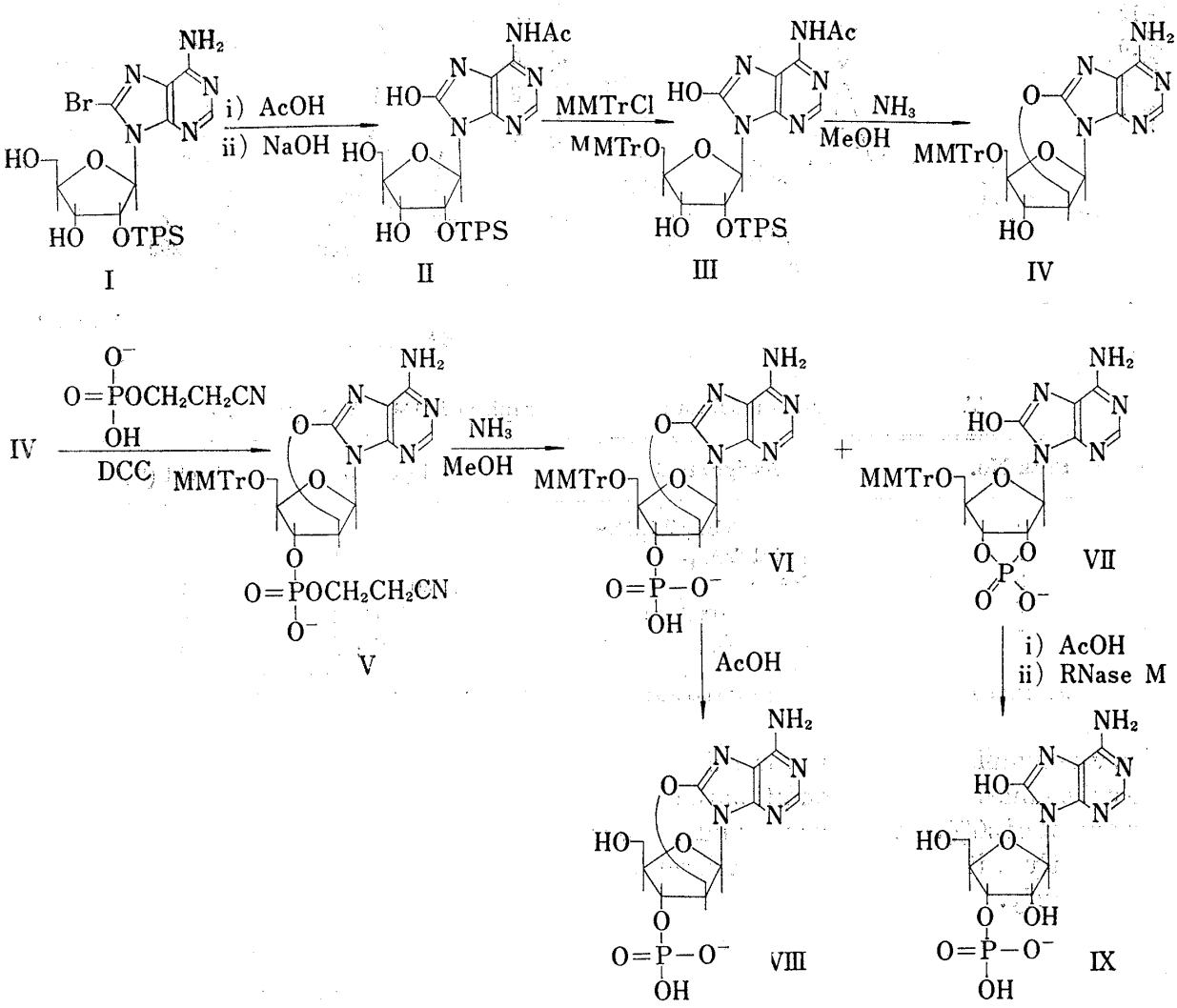

Chart 1

methanol and saturated with dry ammonia gas at 0°. Heating of this solution at 80° for 6 hr gave 5′-monomethoxytrityl-8,2′-O-cycloadenosine (IV) in a yield of 64% calculated from II. UV absorption spectra having $\lambda_{max's}$ 256—261 nm and elemental analysis showed the structure to be correct. The direct tritylation of 8,2′-O-cycloadenosine was avoided because it proceeded in relatively low yields.[1] For the phosphorylation of compound IV, cyanoethyl phosphate and dicyclohexylcarbodiimide (DCC)[8] were employed. When compound IV was allowed to react with 3.5 molar excess of cyanoethyl phosphate and a large excess of DCC in anhydrous pyridine solution at 18—20° for 5 days, the starting material disappeared on paper electrophoreogram. The appropriate work up gave a powder of 5′-O-monomethoxytrityl-8,2′-cycloadenosine 3′-cyanoethyl phosphate (V) in a quantitative yield. Treatment of this sample V with methanolic ammonia at 10—15° for 6 hr and separation of the products by diethylamineethyl (DEAE)-cellulose column chromatography gave results as shown in Fig. 1 and Table I. Material in peak III was almost pure monomethoxytrityl 3′-phosphate (VI). However, the yield was unexpectedly low (27.2%) and a 2′,3′-cyclic phosphate of 8-oxyadenosine (VII) was obtained in a moderate amount (21.7%).

Structure of compound VI and VII was confirmed as follows. Treatment of VI with 80% acetic acid for 1 hr at room temperature gave 8,2′-O-cycloadenosine 3′-phosphate (VIII),

8) G.M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

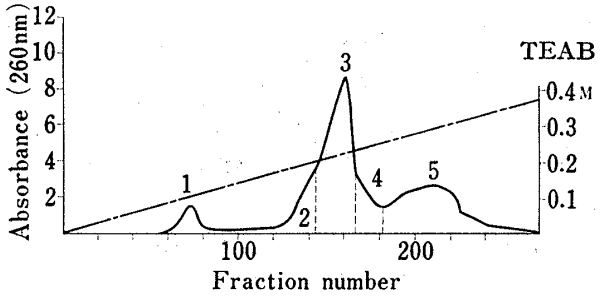

Fig. 1. Column Chromatography of MMTr-A°p

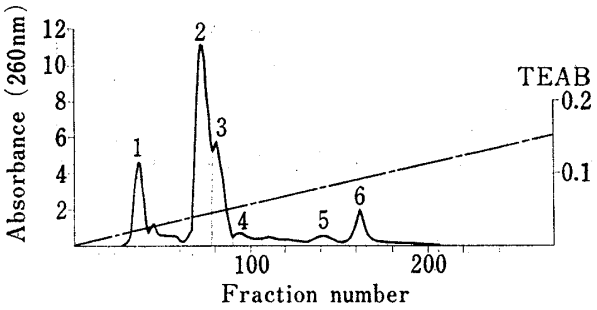

Fig. 2. Column Chromatography of A°pUpG on DEAE-cellulose

Table I. Results of Column Chromatography of MMTrA°p Synthesis

Peak No.	Assignment	TOD_{260} unit	Yield (%)
1	MMTrA°pOCH_2CH_2CN[a]	280	3
2	A°p+MMTrA°p	670	7
3	MMTrA°p	2640	27
4	MMTrA°p+oxy Ap	700(OD_{270})	
5	MMTr-oxy Ap	2070(OD_{270})	22

a) MMTrA°pOCH_2CH_2CN stands for 5′-monomethoxytrityl-8,2′-O-cycloadenosine 3′-cyanoethylphosphate

which was completely resistant toward the hydrolysis with crude snake venom 5′-nucleotidase.[9] Under the same incubation condition the cyclonucleoside 5′-phosphate[1] was dephosphorylated to an extent of 50%. When compound VII was detritylated with 80% acetic acid and digested subsequently with ribonuclease (RNase) M[10] to give 8-oxyadenosine 3′-phosphate (IX) quantitatively.

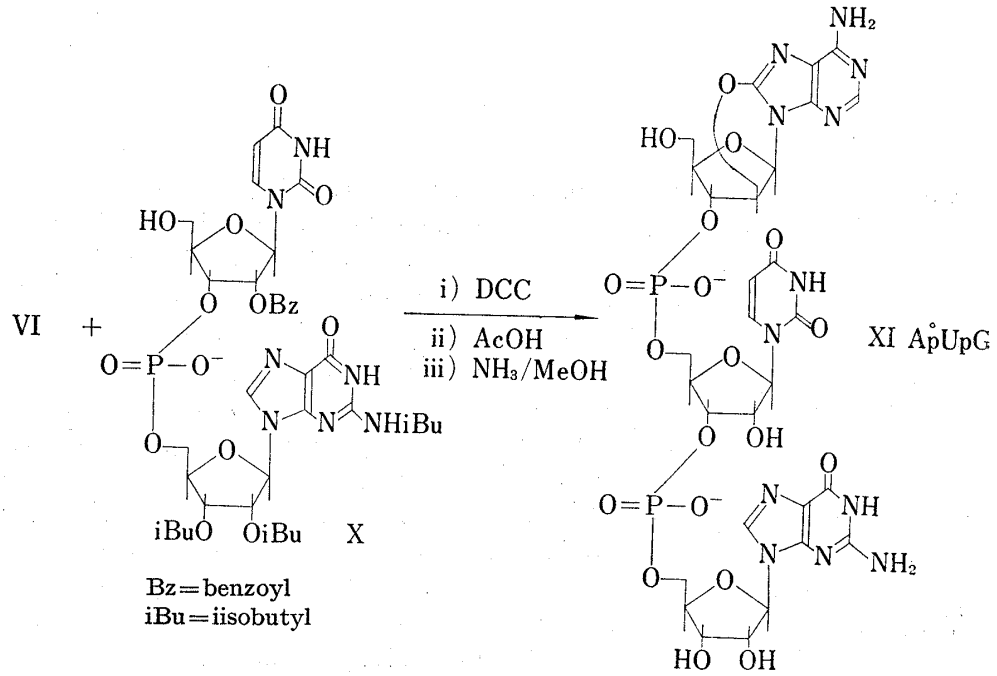

Chart 2

9) Y. Mizuno, M. Ikehara, T. Ueda, A. Nomura, E. Ohtsuka, F. Ishikawa, and Y. Kanai, *Chem. Pharm. Bull.* (Tokyo), **9**, 338 (1961).
10) M. Irie, *J. Biochem.*, **62**, 509 (1967).

Table II. Results of Chromatography of A°pUpG

Peak No.	TOD_{260} unit	Compound	Yield (%)
1	590	A°p-dicyclohexylurea	29
2	1150	UpG	38
3	690	UpG+A°p+X[a)]	
4	110	A°p	5
5	76	X[a)]	
6	200	A°pUpG	4

a) unidentified compound

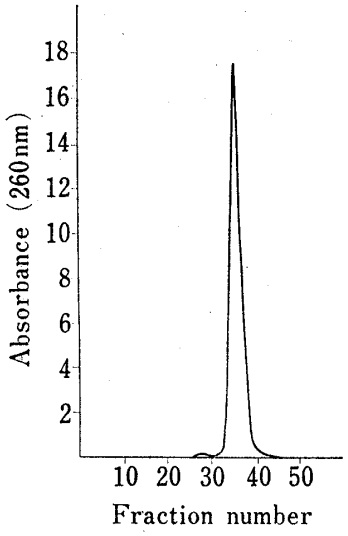

Fig. 3. Gel-filtration of A°pUpG on Sephadex G-15

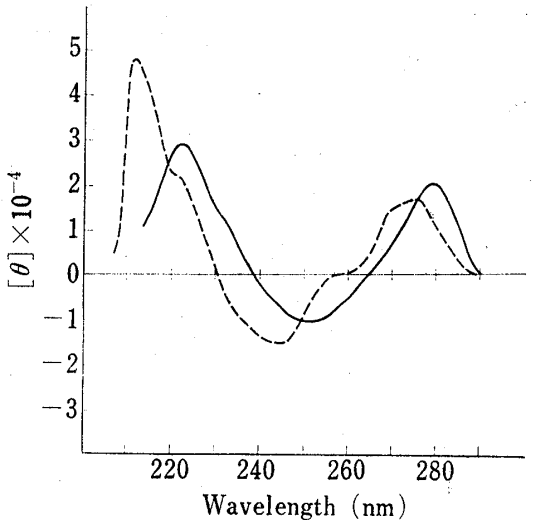

Fig. 4. CD Spectra of 8,2′-O-CycloApUpG
——: taken at 20°, ------: taken at 0°

Compound VI, thus obtained, was finally condensed with 2′-O-benzoyluridylyl-(3′—5′)-N^2,2′,3′-triisobutyryl guanosine[11] (X) (Chart 2) using DCC as the condensing reagent at 25—26° for 6 days. After checking the reaction extent by paper chromatography and paper electrophoresis, the product was deprotected by treatment with acetic acid and NH_3 and applied to a column of DEAE-cellulose. The elution pattern was shown in Fig. 2. The identification of peaks is shown in Table II. The desired product A°pUpG (XI) was obtained from the last peak and yield was 4.1%. The relatively low yield may be due to a conversion of compound VI to a phosphorylurea (XIII) as described later. The material in the last peak was purified by gel filtration on Sephadex G-15 as shown in Fig. 3. The structure of compound XI was confirmed by paper electrophoresis showing a migration distance relative to adenosine 2′,3′-cyclic phosphate 1.06, paper chromatography in three solvent systems (see Table III), and UV absorption properties having λ_{max}'s around 257—259 nm. Digestion with snake venom phosphodiesterase of compound XI gave A°, pU and pG in a ratio of 1.0: 1.1: 0.93 and with pancreatic RNase gave A°pUp and G in 1.1: 1.0. Furthermore, digestion of A°pUp with alkaline phosphatase gave A°pU, which was identical with an authentic sample synthesized separately.[12] Hyperchromicity and hypochromicity calculated after digestion of XI with spleen phosphodiesterase gave values 7.7 and 7.3%, respectively. These values are rather small relative to those of 8,5′-O-cycloadenylyl-(3′—5′)-uridylyl-(3′—5′)-guanosine[3] (XII) (13.6 and 19.1%) and those of natural ApUpG (9.0 and 8.3%). Circular dichroism (CD) spectra of compound XI, XII and ApUpG observed at 0° and 20° are shown in Fig. 4—6. Judging from the shape

11) E. Ohtsuka, "Method in Nucleic Acid Res (A)," Kyoritsu Shuppan Co. Ltd., 1972, p. 106.
12) T. Nagura, unpublished experiment.

Table III. Properties of the Reported Compounds

Compound	Paper chromatography (Rf) Solvent A	B	C	Paper electrophoresis (Rm)[a]	UV spectra λ_{max} (nm) Neutral	H^+	OH^-
MMTrA°p-CE	0.80	0.78		0.68	230 255[b]	258	253
MMTrA°p	0.43	0.61	0.79	1.20	233 257	232 260	257
A°p	0.09	0.19	0.35	1.70	256	259	256
MMTr-HOAp>	0.73	0.85	0.87	0.71	230 270	265 280[c]	280
HOAp>	0.51	0.25	0.54	1.40	259 269	264 282[c]	280
HOAp	0.05	0.17	0.27	1.88	260 270	265 283[c]	280
MMTrA°p-urea	0.82	0.89	0.87	0.00	233 257[b]	231 260	233 256
A°p-urea	0.69	0.71	0.81	0.89	257	260	257.5
A°p-3′,5′-cyclic	0.45	0.44		1.00	257	259.5	257
A°p-pyro	0.08	0.08		1.29	256.5	259.5	257.5
A°pUpG	0.13	0.14	0.27	1.06	257	259	257.5
A°pUp	0.07			1.88	258.5	260	259.5
A°pU	0.31	0.37	0.53	0.80	260	260.5	260

a) relative mobility to Ap
b) measured in 50% ethanol
c) shoulder

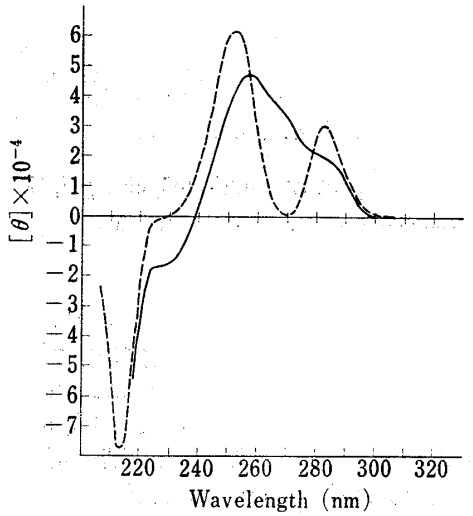

Fig. 5. CD Spectra of 8,5′-O-cycloApUpG
——: taken at 20°, ------: taken at 0°

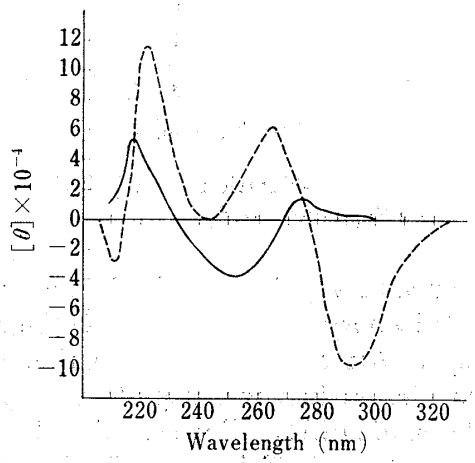

Fig. 6. CD Spectra of ApUpG
——: taken at 20°, ------: taken at 0°

of the curves, conformation change at 0° to 20° of compound XI is rather small, but it seems very large in ApUpG. Compound XII showed a medial change. Curves at 20° of XI and ApUpG are fairly resembled, but that of XII is utterly different. From these observations in UV and CD spectra, it may be deduced that in compound XI each bases situate in a rather freely rotatable unstacked form even at 0°. In contrast to this, the conformation of XII is fairly stable to the thermal disturbance. ApUpG is in a stacked form at 0° and it melts at 20° to an unstacked form.

As shown in Fig. 2, unchanged A°p was not recovered as such, but as a phosphorylurea (XIV) (Chart 3) having one negative charge. From the behaviors in paper electrophoresis and paper chromatography it was thought to be a 3′,5′-cyclic phosphate of A° (XV). However, compound XIV was definitely different from XV synthesized separately from Å 5′-phosphate. Moreover, it differs from a pyrophosphate of A°p (XVI). Thus, 5′-O-monomethoxytrityl-8,2′-O-cycloadenosine 3′-phosphate (VI) was heated in pyridine at 20°, 30° or 50° either in the

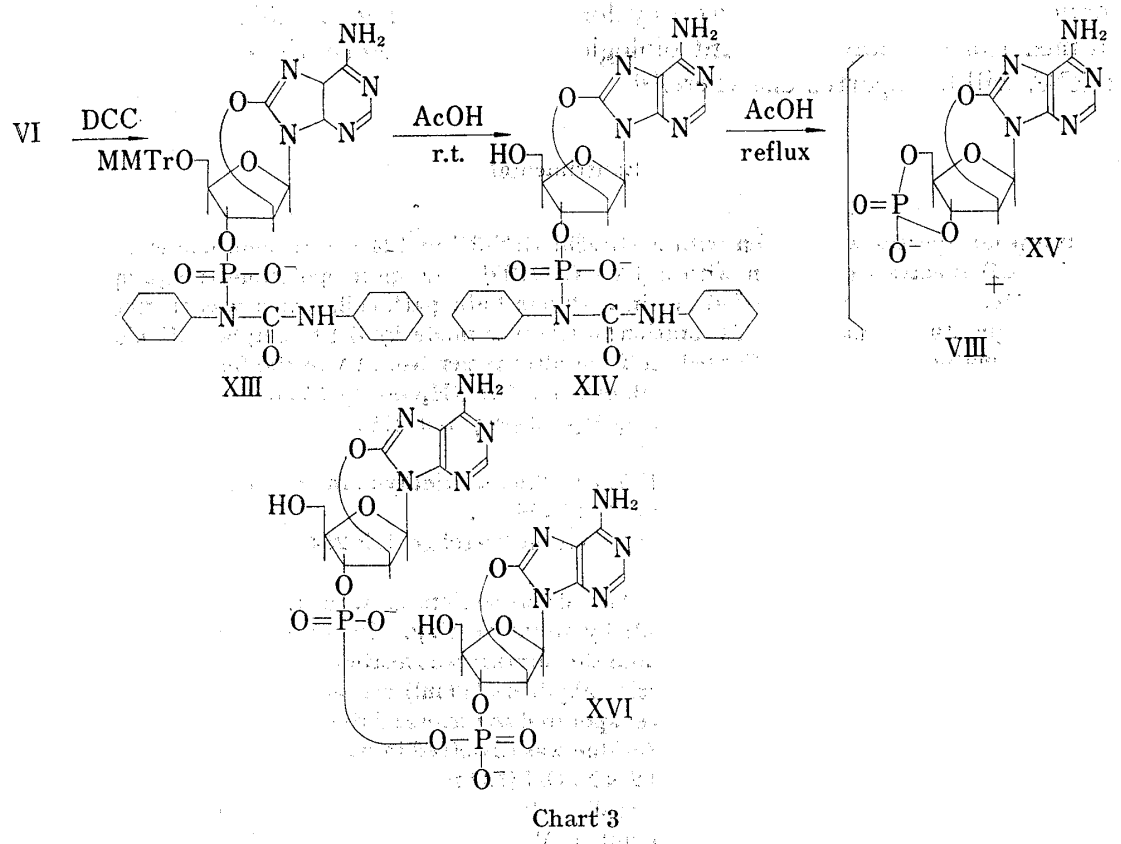

Chart 3

TABLE IV. Results of the Conversion of MMTrA°p in Pyridine

Experiments No.	Temperature (°C)	DCC	A°p (%)	MMTrA°p	MMTr-OHAp>	A°p-urea	Unidentified compound
1	20	none	1.4	90	3.8	0	4.8
2	30	none	0.1	90	2.3	0	5.8
3	50	none	4.1	38	41.5	6	11.7
4	20	10 equiv.				quant.[a]	
5	30	10 equiv.				quant.	
6	50	10 equiv.				quant.	

a) Only a single UV-absorbing spot was detected.

presence or absence of DCC. Results are shown in Table IV. Experiments 1—3 in Table IV show that in the absence of DCC reaction proceeds mainly towards the formation of the 2′,3′-cyclic-8-oxy compound VII;, but in the presence of DCC only compound XIII was obtained. This would suggest that compound XIII may arise from the reaction of MMTrA°p with DCC as observed in the case of uridine 3′-phosphorylurea formation.[13] However, when compound XIII was heated in a mixture of pyridine–acetic acid 1: 1 at 40° for 8 hr, which decomposed uridine 3′-phosphorylurea, no reaction was observed by this treatment. The difference may be ascribed to the absence of vicinal 2′-OH which makes the scission of N–P bond easier. The detritylation of XIII was achieved with 80% acetic acid at room temperature to give compound XIV, which is identical with the compound obtained in peak I of the column chromatography. Refluxing of XIII with acetic acid gave rise to 8-oxyadenosine 3′-phosphate (IX) and 3′,5′-cyclic phosphate of A° (XV). The formation of the latter compound may be another example of activation of the phosphate by P–N bond cleavage.[14]

13) E. Ohtsuka, M. Ubasawa, and M. Ikehara, *J. Am. Chem. Soc.*, **92**, 3445 (1970).
14) M. Ikehara and I. Tazawa, *J. Org. Chem.*, **31**, 819 (1966).

Compound XV is also interesting as a cyclonucleoside analog of 3′,5′-AMP, which is known to be a mediator of many important biological reactions. Activities of A°pUpG as the messenger RNA will be reported elsewhere.[15]

Experimental

UV absorption spectra were taken with a Hitachi EPS-3T or 124 spectrophotometer and summarized in Table IV. CD spectra were taken with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. Measurements were carried out in a 10 mm light path cell, which was thermostated with a JASCO low temperature apparatus. Calibration of CD was made by d-10-camphorsulfonic acid.

Papaer chromatography was performed on Toyo filter paper No. 51A in the following solvent systems: A, 2-propanol–conc. ammonia–water (9: 1: 2). B, ethanol–1 M NH_4OAc (pH 7.5) (7: 3); C, 1-propanol–conc. ammonia–water (55: 10: 35); D, sat.$(NH_4)_2SO_4$–H_2O–2-propanol (79: 19: 2) in descending techniques. Results are summarized in Table IV.

Paper electrophoresis was performed at pH 7.5 in 0.05 M triethylammonium bicarbonate buffer at 35 V/cm for 30 min to 1 hr. Results are summarized in Table V.

Thin-layer chromatography (TLC) was carried out on Kiesel gel HF 254 and eluted with $CHCl_3$–ethanol mixture of appropriate proportion.

N^6-Acetyl-2′-O-TPS-8-oxyadenosine (II)——Anhydrous sodium acetate (1.22 g) was dissolved in glacial acetic acid (30 ml) and acetic anhydride (30 ml) by slight heating. Into the solution 2′-O-TPS-8-bromoadenosine (I)[7] (1.224 g, 2.1 mmoles) was added and the mixture was heated at 130° for 3 hr. Sodium acetate (1.22 g) dissolved in acetic acid (30 ml) and acetic anhydride (30 ml) was added again and the refluxing was maintained for 7 hr. The reaction mixture was evaporated *in vacuo* and the residue was extracted with $CHCl_3$ which was dried on Na_2SO_4 and evaporated. Residue was dissolved in pyridine (100 ml) and water (100 ml). The solution was cooled to 0° with ice-water and 2 N NaOH (100 ml) was added. After 5 min at this temperature, the whole was neutralized with 1 N HCl at 0°. Precipitated material was collected by decantation and dissolved in $CHCl_3$ containing a small amount of MeOH. Addition of NaCl to the supernatant gave a white powder, which was collected by filtration. The $CHCl_3$ solution was washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated. The residue was combined with the white powder and washed with acetone and ether. Yield 0.92 g (80%). UV: $\lambda_{max}^{H^+}$ 288.5 nm, $\lambda_{max}^{H_2O}$ 288.5 nm, $\lambda_{max}^{OH^-}$ 309.5 nm. This material showed one spot on TLC.

5′-O-Monomethoxytrityl-8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (IV)——The 2′-TPS-8-oxy compound (II) (1.0 g, 1.69 mmoles) was dissolved in anhydrous pyridine (30 ml) and monomethoxytrityl chloride (0.625 g, 2.03 mmoles) was added. After heating the solution at 40° for 24 hr, monomethoxytrityl chloride (0.30 g) was added again and the heating was continued for 10 hr. The reaction mixture was poured into ice-water (200 ml) containing 2% ammonia. The solution was extracted with $CHCl_3$ on addition of saturated NaCl solution. The $CHCl_3$ layer was dried on Na_2SO_4 and evaporated *in vacuo*. A pale yellow oil was obtained. The oily residue was dissolved in anhydrous methanol (30 ml), which was saturated with NH_3 gas and heated at 80° for 6 hr in a seeled steel tube. Methanol was carefully evaporated and the residue was dissolved in ethanol by slight heating. After decolorizing with charcoal the solution was stored in a refrigerator for crystallization. Compound IV, mp 184—186°, was obtained in a yield of 0.575 g (64%). UV: $\lambda_{max}^{H^+}$ 233.5, 261 nm, λ_{max}^{EtOH} 235, 257 nm, $\lambda_{max}^{OH^-}$ 232, 256 nm. TLC showed one spot.

5′-O-Monomethoxytrityl-8,2′-O-cycloadenosine 3′-Cyanoethyl Phosphate (V)——MMTr-8,2′-O-cycloadenosine (IV) (300 mg, 0.57 mmole) was made anhydrous by evaporation twice with added pyridine and dissolved in pyridine (20 ml) with pyridinium cyanoethyl phosphate (2.0 mmoles) and DCC (588 mg, 2.89 mmoles). The reaction mixture was kept at room temperature (18—20°) for 5 days. After test of the reaction extent by paper electrophoresis, 50% aqueous pyridine was added to the mixture at 0°. Dicyclohexylurea which precipitated was filtered off, the filtrate was extracted with *n*-pentane (20 ml × 3) and evaporated *in vacuo*. The residue was extracted with a *n*-BuOH–H_2O mixture, which was washed twice with water. Butanol layer was evaporated *in vacuo*. The residue was dried by evaporation with pyridine and was dissolved in pyridine. Adding the pyridine solution dropwise into a mixture of ether–*n*-pentane (1: 1) gave a powder, which was collected by centrifugation. Yield was 0.417 g (99.5%).

5′-O-MMTr-8,2′-O-Cycloadenosine 3′-Phosphate (VI) and 5′-O-MMTr-8-oxyadenosine 2′,3′-Phosphate (VII)——Compound V, obtained as above, (0.417 g) was dissolved in methanol saturated with NH_3 at 0° (20 ml) and kept at 10—15° for 6 days. Methanol was evaporated at low temperature and the residue was dissolved in a water–pyridine mixture, which was applied to a column (3.1 × 41.5 cm) of DEAE-cellulose (bicarbonate form). The column was eluted with a linear gradient of 0.4 M triethylammonium bicarbonate in 20% ethanol (3 liters) and 20% ethanol (3 liters). One fraction (20 ml) was collected every 14 min, The

15) K. Shimokawa, T. Nagura, E. Ohtsuka, and M. Ikehara, *Seikagaku*, **45**, 485 (1973).

elution pattern was shown in Fig. 1. The yield and properties of nucleotides appeared in peak 1—5 are summarized in Table I and III.

Enzymatic Hydrolysis of Compounds VI and VII——i) A small amount (*ca.* 10 optical density $(OD)_{260}$ units) of VI was treated with 80% AcOH at room temperature for 1 hr, solvent was evaporated *in vacuo*, and applied to a paper chromatography in solvent C. A spot corresponding to A°p was extracted (*ca.* 1 OD) and incubated with crude snake venom phosphodiesterase (5 mg/ml) 20λ and 1 M NH_4HCO_3 (pH 9.0) 20λ in total volume of 50λ adjusted with H_2O at 37° for 16 hr. The whole was applied to paper chromatography in solvent B. VI was not hydrolyzed. A° 5′-phosphate[9] was hydrolyzed to an extent of 50% in this condition. *Rf* of A°p was 0.39 (D), 0.17 (B) and of A°p was 0.38 (D), 0.19 (B).

ii) A small amount (*ca.* 10 OD_{260} units) of compound VII was treated with 80% AcOH at room temperature for 1 hr. Residue obtained by the evaporation of AcOH was applied to a paper chromatography in solvent B. A spot corresponding to 8-oxyadenosine 2′,3′-cyclic phosphate (3 OD units) was incubated with RNase M (2.5 mg/ml) 20λ and 1 M $AcONH_4$ 30λ in total volume of 100λ adjusted with H_2O at 37° for 12 hr. Application of the mixture to a paper chromatography (B) and paper electrophoresis showed spots (*Rf* 0.18 and R_{Ap} 1.8, respectively) corresponding to 8-oxyadenosine 3′-phosphate. Thus complete hydrolysis of VII to 3′-phosphate was confirmed.

8,2′-Anhydro-8-oxyadenylyl-(3′—5′)-uridylyl-(3′—5′)-guanosine (XI)——Compound VI (pyridinium form, 110 mg, 0.136 mmole) and 2′-O-benzoyl-uridylyl-(3′—5′)-N^2,2′,3′-triisobutyryl guanosine[11] (X) (pyridinium form, 134 mg, 0.136 mmole) were dissolved in pyridine (10 ml) and evaporated to remove a trace of water. This procedure was repeated 3 times. Finally the residue was dissolved in pyridine (10 ml), which was concentrated to 3 ml *in vacuo*. DCC (280 mg, 1.36 mmoles) was added and the mixture was kept at 25—26° for 6 days in the dark place. To the reaction mixture was added 50% pyridine under cooling by ice-water bath. Dicyclohexylurea was filtered and washed with 50% pyridine. Filtrate and washings were combined and evaporated several times with added pyridine. The final pyridine solution was poured dropwise into *n*-pentane to bring about precipitation. The precipitates were dissolved in 80% acetic acid (20 ml) and kept at room temperature for 1 hr. Acetic acid was evaporated *in vacuo*, the residue was coevaporated with addition of water and finally with pyridine three times. To the residue was added methanolic ammonia (30 ml) and kept at 15° for 15 hr. Methanol was carefully evaporated and the residue was dissolved in H_2O (500 ml). The solution was applied to a column (1.0×44 cm) of DEAE-cellulose (bicarbonate form), which was eluted with 0.2 M triethylammonium bicarbonate (3 liters) and H_2O (3 liters) in a linear gradient technique. One fraction (17.2 ml) was collected per 12.3 min. Elution pattern was shown in Fig. 2 and the yield of products was listed in Table IV. A°pUpG obtained in peak 6 was applied to a Sephadex G-15 column and eluted with 0.05 M triethylammonium bicarbonate buffer (pH 7.5). One fraction (3 ml) was collected in 25.7 min. Elution pattern is shown in Fig. 3. A°pUpG appeared at tube No. 33—38 was combined, evaporated *in vacuo* and further evaporated three times with methanol. A°pUpG was obtained in a yield of 156 OD_{260} units. Properties are listed in Table III.

Enzymatic Digestion of A°pUpG (XI)——i) Compound XI (*ca.* 3 OD_{260}) dissolved in H_2O (120λ) was incubated with snake venom phosphodiesterase (1 mg/ml) 10λ in the presence of 1 M $(NH_4)_2HCO_3$ 20λ at 37° for 10 hr. Application of the mixture to a paper chromatography in solvent B showed spots corresponding to A° (*Rf* 0.12), pU (*Rf* 0.21) and pG (*Rf* 0.63). Each spot was extracted with water (1.5 ml) and UV absorption was measured. A°: pU: pG=1.0: 1.1: 0.93.

ii) Compound XI (*ca.* 5 OD_{260}) was incubated with pancreatic RNase (2 mg/ml) 10λ, and 1 M NH_4OAc 20λ in total 100λ H_2O at 37° for 8 hr. Application of the mixture to a paper chromatography, which was developed in solvent C showed two spots corresponding to A°pUp (*Rf* 0.07) and G (*Rf* 0.39) in a ratio of 1.1: 1.0. A°pUp (*ca.* 2.5 OD_{260}) was extracted with water and incubated with sheep intestine alkaline phosphatase (2 mg/ml) 10λ and 0.1 M Tris-HCl (pH 7.8) 20λ in total volume of 100λ at 37° for 4 hr. Paper chromatography of the product in these solvent systems gave *Rf* 0.31 (A), 0.38 (B) and 0.55 (C), which were identical with those of A°pU synthesized separately.

Table V. Properties of ApUpG and Its Analogs

Compound	Hyperchromicity (%)	λ max (nm)	Hypochromicity (%)	λ max (nm)
8,2′-O-cycloApUpG	7.7	257	7.3	257
8,5′-O-cycloApUpG	13.6	258.5	12.1	259.5
ApUpG	9.0	258	8.3	258

Hyperchromicity and Hypochromicity of XI, A°pUpG and ApUpG——Each trimer (3—4 OD_{260} units) was digested with phosphodiesterase as above and diluted with 0.05 M phosphate buffer (pH 7.0) containing 0.1 M KF (5 ml). Results are listed in Table V.

CD Spectra of XI, A°pUpG and ApUpG——Nucleotide concentration was 1.5—1.58 OD_{260}/ml. 0.1 M KF and 0.05 M phospoate buffer (pH 7.0) were used. Spectra taken at 0° (·····) and at 20° (——) are shown in Fig. 4—6.

5′-MMTr-A° 3′-Phosphoryl-dicyclohexylurea (XIII)——Compound VI (3 mg) was heated at 20—50° in pyridine (0.05 ml) either in the absence or in the presence of DCC (7.8 mg, equiv.). Results are listed in Table IV. Properties of the products were listed in Table III. When MMTr-phosphoryl urea (XIII) was treated with 80% acetic acid at room temperature, MMTr group came off and compound XIV was obtained. At the reflux temperature for 8 hr, 3′,5′-cyclic phosphate of A° (XIV) and compound VIII were obtained.

8,2′-O-Cycloadenosine 3′,5′-Cyclic Phosphate (XV)——8,2′-Cycloadenosine 3′-phosphate (VIII) (5 mg) was dissolved in 50% pyridine. Morpholino-N,N′-dicyclohexylcarboxyamidine[16] (5 mg) was added to the solution and pyridine was evaporated. After evaporation several times with addition of pyridine, the residue was dissolved in pyridine (2 ml) and DCC (10 mg) was added. Refluxing of the solution for 8 hr gave A°-3′,5′-cyclic phosphate with 8-oxyadenosine 2′,3′-cyclic phosphate in 1 : 1 ratio. Properties are listed in Table V.

8,2′-O-Cycloadenosine 3′,3′-Pyrophosphate (XVI)——Compound VIII (5 mg) was dissolved in pyridine, morpholino-N,N′-dicyclohexylcarboxyamidine (3 mg) was added and evaporated twice with pyridine. The residue was dissolved in pyridine (2 ml) and heated with DCC (5 mg) at 50° for 2 hr. The reaction mixture was extracted with *n*-pentane, the water layer was evaporated, and the residue was dissolved in pyridine. Precipitates appeared by addition of the pyridine solution into *n*-pentane was collected by centrifugation. Properties of compound XVI are listed in Table III.

16) J.G. Moffatt and H.G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).